A STUDY ON THE EFFECTS OF EYESTALK ABLATION ON THE ELECTROPHORETIC PATTERNS OF GENERAL PROTEIN IN *PENAEUS INDICUS* H. MILNE EDWARDS

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CERTIFICATE

This is to certify that this Dissertation is a bonafide record of the work carried out by **Shri. Surendra Kumar Ghadei** under my supervision and that no part thereof has been presented before for any other degree.

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PREFACE

Among the penaeid prawns available for culture in Indian waters the present stress is on the Indian white prawn Penaeus indicus H. Milne Edwards due to its wide distribution along both the coasts, high market demand and vast amount of information available on their biology and seed distribution. Although P. monodon grows to a larger size P. indicus is equally a good candidate species as its life history stage is very short and good survival rates.

In order to bring out another 50000 ha of available brackishwater area in coming years under prawn culture programme in India, the availability of prawn seeds is of fundamental importance. As the traditional seed collection is highly fluctuating in nature, most farmers can not have more than one crop in a year. Induction of gonadial maturation in pond grown stocks of selected species now appears to be the best method to ensure hatchery production of prawn seeds and regular supply of seeds.

In hatcheries, seeds of P. indicus are produced by subjecting the adult female P. indicus to ablation of any one of the eyes and feeding them with nutritious natural/artificial food to induce gonadial maturation and spawning. Such induced spawning is naturally preceded by changes in the neuroendocrine control of the process of reproduction. In this context

basic information on the biochemical changes manifested in the ovaries during maturation is very essential. The complex process of accumulation, release and transport of nutrient reserves from the storage organs to gonads, metabolic capacities of organs participating in the reproductive cycle, regulation and coordination involved in achieving spawning and allied biochemical aspects are yet to be explained in order to explore and exploit the full potential of prawn culture, from the stage of artificial production of seeds.

As the proteins form the structural units of tissues/organs, quantitative and qualitative changes in the protein systems are also expected to occur during the induced spawning process. A knowledge of such changes may be of fundamental importance, in perfecting the eyestalk ablation technique for induced spawning. Hence the present electrophoretic investigation was undertaken to visibly reveal the effects of eyestalk ablation on the general proteins in P. indicus.

The main objectives of the present study were:

(1) Electrophoretic detection and demonstration of the nature and quantity of proteins in Hepatopancreas (HP), hemolymph (HM), Ovary (G), Nervous system (N), Eye (E), Antenna base (A), and Body muscle (M) in Penaeus indicus before eyestalk ablation.

(2) A comparison of electrophoretic patterns of general proteins obtained before and after eyestalk ablation to evaluate the effects of eyestalk ablation on the quantity and quality of proteins in different tissues of P. indicus.

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INTRODUCTION

In the realm of brackishwater aquaculture, more accent and thrust are given towards penaeid prawn farming due to its export potential, high market demand and better returns on investment. Out of total sea food export of India, prawns alone contribute around more than 80% by value and 60% by volume. Hence prawn is considered as pinkish gold of the sea by virtue of its high unit value.

Penaeid prawns occupy a pride of place both in capture and culture fisheries of India. The prawn production from the currently exploited inshore fishing grounds is stagnating for the last few years. Raising prawn through traditional farming in the brackishwater areas has been recognised as a viable alternate proposition for augmenting export quality prawn production The market demand and unit value commanded by shrimp in International market has motivated the adoption of so called coastal/brackishwater prawn culture technology with a view to generating increased production of the commodity.

Timely supply of adequate number of quality prawn seeds is of fundamental importance to any organised successful prawn farming programme A limited quantity of wild seeds can be collected from selected pockets of the Indian coast. But large number of seeds required for the operation

of intensive, semi-intensive type of commercial prawn farming can not be obtained from this natural source alone. Besides, seed availability from natural sources will fluctuate from season to season, from year to year and even from centre to centre. Thus bulk supply of wild prawn seeds for large scale farming operations become difficult. It also takes several days for accumulation of sufficient quantity of seeds and considerable mortality can occur during storage before stocking. These problems necessiate the establishment of prawn hatcheries in the coastal belt to support commercial prawn farming. Penaeid prawn hatcheries have been developed in many countries to meet the demand of quality prawn seeds for aquaculture. Technology for producing shrimp seed in the hatchery through induced breeding under controlled conditions and mass rearing upto stockable size is now available in several parts of the world. However in India, establishment of prawn hatcheries is in the developing stage.

A steady supply of spawners is essential for efficient programming of the hatchery operations. The collection of spawners from the sea is a costly, cumbersome and uncertain affair. Although all the cultivable species of penaeid prawns grow in brackishwater ponds, the females do not attain sexual maturity in the same environment. In nature the adults migrate from the brackishwaters to the sea where they attain sexual maturity before spawning. Hence the timely availability of the fully ripe spawners of the desired species from the sea, the high cost of their fishing and transporting them alive to the hatcheries are some of the main constraints in the development and management of hatcheries for large scale culture

operations. To overcome these constraints research is being undertaken in many countries to develop an artificial maturation technology using captive immature prawns of specific sizes and ages. The most successful technology of induced maturation of prawn in captivity is known as "eyestalk ablation" involving removal of the eyestalk which holds the location of ovary inhibiting hormone (OIH). The eyestalk ablation leads to induced maturation and spawning.

Eyestalk ablation technology was adopted from the classical experiments of Eversince Panouse (1943) on the female shrimp, Leander serratus, demonstrating for the first time that removal of the eyestalks (as source of the OIH) during sexual inactivity led to rapid increase in ovarian size and precocious egg deposition. The technology has been elaborated over the last 20 years in various crustaceans such as Pandalus hessleri (Aoto and Nishida, 1956), Carcinus maenas (Demeusy, 1965), Scylla serrata (Ranganekar and Deshmukh, 1968), Paratelphusa hydrodromous (Adiyodi and Adiyodi, 1970), Palaemon paucidens (Kamiguchi, 1971), Penaeus duorarum (Idyll, 1971; Caillouet, 1973), Crangon crangon (Bomirski and Klek, 1974), Palaemon serratus (Ryckaert et al.,1974), Penaeus japonicus (Laubier, 1975), Orconectes gammarellus and Lysmata seticaudata (Charniaux-cotton, 1975), Penaeus orientalis and Penaeus monodon (Arnstein and Beard, 1975) Penaeus aztecus (Aquacop, 1975), Penaeus merguiensis (Alikunhi et al., 1975; Nurijama and Yang, 1977), Uca pugilator (Webb, 1977), Penaeus monodon (Aquacop, 1977a, 1979, 1982; Santiago,1977; Chen, 1977; Primavera, 1978a,b,1982; Primavera and Borlangam, 1977;

Primavera et al., 1978, Halder, 1978; Beard and Wickins, 1980; Tiensongrasmi, 1980; Muthu and Laxminarayana, 1971; Rajyalakshmi et al., 1987), Penaeus indicus (Muthu and Laxminarayana, 1977, 1979, 1981), Penaeus kerathuras (Lumare, 1979), Penaeus plebejus (Kelemec and Smith, 1980). Dall (1965b) in Metapenaeus spp. and Nakamura (1974) in Penaeus japonicus studied the neurosecretory structures and highlighted it's general resemblance to the other group of crustaceans in it's organisation. Among the Indian penaeid prawns Metapenaeus monoceros (Madhyastha and Ranganekar, 1976), Penaeus monodon (Nanda and Ghosh, 1985) and Parapenaeopsis stylifera (Nagabhushanam et al., 1986) are the few members, which received brief attention, as far as the neurosecretory structures are concerned.

The mechanism of induced maturation and somatic growth in crustaceans is controlled and regulated by hormones. The role of hormones in the mechanism of reproduction of penaeid prawns is not fully understood but is likely to be similar to that of the other decapod crustaceans as discussed by Adiyodi and Adiyodi (1970, 1974). Egg production in prawns, as in other crustacea, is a cyclic process under the hormonal control of the neurosecretory centres. Among these centres, the "X-organ sinus gland complex" in the eyestalk close to cornea seems to produce an ovary inhibiting hormone (OIH) which inhibits vitellogenesis (Carlisle and Knowles, 1959; Waterman, 1969; Green, 1967) while the centres in the brain and thoracic ganglia appear to produce an ovary stimulating hormone (OSH) which promotes vitellogenesis (Kulkarni and Nagabhushanam, 1980; Nagabhushanam and

Kulkarni, 1982). During the quiescent phase of the ovary the X-organ seems to produce a high titre of ovary inhibiting hormone (OIH) which restrains vitellogenesis either directly or through its action on the neurosecretory centres which produce the OSH. In nature when the physiological and environmental factors ie., high salinity, high pressure (depth), low intensity of light (darkness) are conducive to reproduction, the titre of the OIH secreted by the X-organ complex is probably reduced, thereby allowing vitellogenesis to take place under the influence of OSH. On the basis of this hypothesis the technique of eyestalk ablation has been evolved for inducing the penaeid prawns to mature in captivity. By the removal of one eyestalk, the titre of the OIH is arbitrarily reduced and this leads to ovarian development.

Reproductive activity and somatic growth in decapods crustacea are antagonistic in nature. Female reproduction alternates with moulting and occurs during the intermolt period. The X-organ shows histological cycles associated with oocyte development and in some isopods ovariectomy causes hypertrophy of the sinus gland, this provides supporting evidence for relationship between the ovaries and the eyestalk neurosecretory system. The bihormonal theory of ovarian control is based on the principle that the ovarian growth in the crustacea is generally balanced through the antagonistic actions of OIH and OSH (Adiyodi and Adiyodi, 1970). After reaching puberty, the decapods generally develop gametes during their intermolt period. The postmolt period is concerned with the formation of new exoskeleton and tissue growth whereas the premolt period involved in

preparation for the next molt in adults (Passano, 1960).

Both molting and reproduction require mobilization of organic reserves from storage sites to the epidermis and gonads. This cyclic mobilization of reserves is coordinated hormonally. In juvenile, somatic growth and ecdysis take precedence over ovarian development where as in mature individuals reproductive growth is of greater importance. Thus according to the developmental stage of an individual, eyestalk removal will accelerate which ever process is currently dominant. Thus MIH and OIH act synergestically during postmolt and antagonistically during intermolt phases when reproductive development occurs. It has been seen that highest OIH activity was found in the eyestalks of the prawns having inactive and spawned ovary (Kulkarni and Nagabhushanam, 1979).

There are 2 types of eyestalk ablation: (1) Unilateral (single) eyestalk ablation. (2) Bilateral (both) eyestalk ablation.

There are 8 different methods available for eyestalk ablation:

(1) Cutting the eyestalk near the base with a pair of scissors (Arnstein and Beard, 1975; Lumare, 1979).

(2) Scissor cutting followed by cauterisation with pencil type soldering iron (Caillouet, 1973).

(3) Pinching of eyestalk (Aquacop, 1977).

(4) Ligation of eyestalk with a thread.

(5) *Squeezing the eyeball contents out* (Rodriguez, 1979).

(6) Incision of eyeball with a blade/razor followed by squeezing out the contents and crushing the eyestalk (Primavera, 1978a, b).

(7) Incision of eyeball with a blade/razor followed by enucleation of contents (Kelemec and Smith, 1980).

(8) Electrocauterisation (Muthu and Laxminarayana, 1979, 1981).

Bilateral eyestalk ablation, although leads to rapid ovarian growth, does not result in spawning, the ova get reabsorbed without being released from the ovary (Caillouet, 1973; Duronslet et al., 1975; Aquacop, 1975; Wear and Santiago, 1976). The unilateral eyestalk ablation successfully leads to ovarian maturation and subsequent spawning (Arnstein and Beard, 1975, Wear and Santiago, 1976). The first successful maturation and spawning of viable eggs in Penaeus indicus by single eyestalk ablation was reported by Muthu and Lakshminarayana (1977, 1979 and 1980). They used electro-cautery apparatus to remove the eyestalk which seals the cut instantly, avoiding blood loss and ensures cent percent survival. A study on the induced breeding of Penaeus monodon using unilateral eyestalk ablation has been reported by Halder (1978) in Bakkhali fish farm of West Bengal.

Eyestalk also plays an important role in the control and regulation of other physiological functions. This is due to the presence of other

neurosecretory hormones in the eyestalk. These functions are revealed by studying the effects of eyestalk ablation on various physiological aspects of the organism. The eyestalk apart from the OIH also carries a number of neurosecretory hormones that regulate lipid metabolism and protein synthesis in the hepatopancreas, calcium metabolism during cuticle formation and induce hyperglycaemia in the hemolymph to combat stress. They also affect the water balance during ecdysis and inhibit production of molting hormone by the Y-organ and influence the movement of pigments in the chromatophores (Hignam and Hill, 1978). In decapods, the eyestalk factors seem to regulate the deposition and subsequent mobilization of organic reserves utilized for reproduction (Adiyodi, 1969; Yamamoto, 1960; Miyawaki and Tanoue, 1962). Free sugars in hepatopancreas of the Paratelphusa hydrodromous fluctuate during the ovarian cycle; these same sugars are found during early vitellogenesis, apparently being utilised in the formation of lipoprotein (Adiyodi and Adiyodi, 1970). The eyestalk also contains substances that raise blood sugar levels in crabs (Telford, 1968). The hepatopancreas also stores carbotoproteins that are mobilized to the ovary during vitellogenesis (Goodwin, 1951). Adiyodi (1968a,b), reported that a female Specific Protein (FSP) is formed almost exclusively during vitellogenesis in crabs. Its formation is apparently influenced by the reproductive hormones. In crabs, FSP accumulates during the postmolt and reaches a maximum in hemolymph before onset of vitellogenesis.

Evidence for the production of the eyestalk factors influencing incorporation of aminoacids into protein has been reported by Gorell and

Gilbert (1970), Mc Whinnie and Mohrherr (1970) and Raghavaiah (1977). Substantial synthesis of protein during the intermolt period, between ecdyses and the incorporation of lebelled aminoacids into protein have been studied by Skinner (1965, 1966a,b), Kurup and Scheer (1966), Yamaoka and Raghavaiah (1977). A large amount of work has been done on the metabolism of carbohydrate and lipid in crustaceans (Hohnke and Scheer, 1970). Lipid, protein and carbohydrate metabolism seem to be under hormonal and endocrine control. Eyestalk ablation in crustaceans usually results in precocious gonadal development. Therefore putative target tissues of the OIH probably respond to eyestalk ablation by a rapid increase in biosynthetic activity of yolk proteins (Vitellogenin). It is also known that yolk proteins produced in extra ovarian tissues must be transported to the ovary through hemolymph (Adiyodi, 1985; Derelle et al., 1986; Fielder et al., 1971; Nelson et al., 1988; Yano, 1987). The ovary and the hepatopancreas dramatically increased their incorporation of lebelled leucine into proteins after the eyestalk ablation of Penaeus vannamei.

Depending on the species and their current molt stage the response of the reproductive and other organs to eyestalk ablation is variable (Quackenbush, 1986). In the Shrimp P. vannamei the hepatopancreas does not appear to store or accumulate egg yolk proteins. In fact in other shrimp of the same group, it is suggested that the hepatopancreas does not significantly contribute to egg yolk production (Tom et al., 1987; Yano, 1987). In P. vannamei the hepatopancreas can produce a subunit of egg yolk protein and its production is inhibited by eyestalk factors (Quackenbush,

1986). Quantities of proteins, carbohydrates and fat contents in the prawn P. indicus, proteins, lipids and glycogen in bivalve Perna spp. varied at different maturity and experimental conditions (Asokan, 1983; Ranganekar et al., 1961).

Gel electrophoresis techniques have recently been applied to studies in aquaculture and breeding programme of economically important fishes and prawns (Hedgecock et al., 1976; Moav. R et al., 1976). Gel electrophoresis methods have enabled research workers in various field of biology to visualise different kinds of proteins and their variants present in many organisms (Davis, 1964; Tsuyuki and roberts, 1963; Jones and Mackie, 1970; Dhulkhed and Rao, 1976; Kulkarni et al., 1980; Thomas, 1981, Prathiba, 1984, Philiph, 1989). Disc electrophoresis showed certain protein patterns in ovary, hemolymph, hepatopancreas of the hermit crab C. longitarsis (Natarajan, Khan and Reddy, 1975).

Disc gel electrophoresis indicated different protein patterns in hepatopancreas, ovary and hemolymph of crabs in different stages of molt cycle and reproductive activity (Adiyodi and Adiyodi, 1970).

Little information is available on the relationship between the eyestalk and the qualitative nature of protein storage in different organs/ tissues, its mobilization of ovary in the Indian penaeid prawn Penaeus indicus. Such basic informations, on the proteins contained in different tissues and also under the control of eyestalk hormones may be made useful in understanding better the mechanism of maturation induced by eyestalk ablation.

Hence the objective of the present short term investigation was to study the effects of eyestalk ablation on the electrophoretic patterns of general protein in selected tissues of Penaeus indicus.

MATERIALS AND METHODS

GENERAL ASPECTS

1. SOURCES OF EXPERIMENTAL ANIMAL

The Indian white prawn Penaeus indicus H. Milne Edwards was selected for the present study. It is found all along the coasts, but more abundant in the South-west coast and South-east coast of India and also forms a considerable fishery in the estuaries and backwaters. The male and female attain length of 128mm and 143mm at the end of the first year and 163 and 173 mm at the end of the second year respectively in the open sea. The P. indicus is known to breed and undergo early development in the open sea. The size at first sexual maturity of female is about 130.2 mm and fecundity varies from 68000 to 731000. The peak spawning season in Cochin area is October - November and May - June.

2. COLLECTION OF EXPERIMENTAL ANIMALS

Specimens of P. indicus 130 ± 10 mm in total length and 20 ± 2 gm in body weight were collected from the "Thoppilikettu" prawn filtration pond at Edavanakad near Narakkal. They were caught by prawn filtration bag net. The females were in immature stage with a thin, translucent and unpigmented ovary confined to the abdomen. Some of the males were in mature stage with unification of petasma and well developed spermato-

phores at the base of the fifth walking legs. One brood female prawn was collected from open sea at a depth of 30 m by trawl net. It was in late maturing stage with light green ovary visible through exoskeleton **[Plate-1a].**

3. ACCLIMATIZATION OF THE EXPERIMENTAL ANIMALS TO LABORATORY CONDITIONS

The animals collected from culture pond were brought to the laboratory in plastic buckets, lined with polythene sheet and periodical flushing of water with hand. Upon arrival in the laboratory, the animals were gradually acclimatized to higher salinity in holding tanks since they were in 5 ± 2‰ saline water medium when collected. By keeping the animals 12 hours each in 10‰, 15‰ saline water and 6 hours each in 20‰ 25‰ and 30‰ saline water, they were brought to fully saline water of 33‰ in maturation tank. As the quality of water in maturation tank has a profound influence on the maturation of the penaeid prawns, the good quality high saline water was collected from Saudi and Manassery towards south of Fort Cochin. The salinity of water was 33.7‰, very close to that of open sea water in which penaeid prawn attains full maturity and spawning. Once the animals were recovered from the transport stress they were transferred from holding tank to maturation tank for experiment.

EXPERIMENTAL SET UP

1. MATURATION TANK

The size of FRP maturation tank was 0.9 x 0.6 x 0.6 m with

seawater upto 0.45 m and bottom of tank filled with 5 cm thick sand, which acts as a filter bed for entrapment of sediments. In addition to this, a 20 cm dia airlift subgravel filter, connected with 3 electrically operated aerators were fitted to the tank. The inner side of the tank was smooth enough, so that the prawns did not get injured by abrasion **[Plate-1b].**

2. AERATION

Aeration was provided by the help of 5 electrically operated aerators per tank in maintaining sufficient dissolved oxygen concentration in the water, ensuring even water temperature throughout the water column through turbulence and reducing level of ammonia content in the water.

3. FEEDING

The prawns were fed ad libitum with fresh clam meat @ 20% of body weight per day (2 bigger size clams/prawn/day).

4. MAINTENANCE OF ANIMALS

Care was taken to maintain the water qualities like temperature (27 ± 1°C), dissolved oxygen 5 ± 1 mg/ml and pH 8.2 ± 0.2. The uneaten food and fecal matter was siphoned out everyday. Everyday the pH of the water was maintained around 8.2 ± 0.2 by adding anhydrous Na_2CO_3 @ 2 gm/ litre because pH causes maturation of gonad. The required quantity of Na_2CO_3 was first dissolved in freshwater and then added to seawater.

Then the water was thoroughly mixed and allowed to remained for 2 days without disturbance. In this way the undissolved Na_2CO_3 particles were settled at the bottom. Only the upper clear water was taken and added to maturation tank which contains experimental animals. Otherwise the undissolved Na_2CO_3 particles will enter to gill region and can cause mass mortality (personal observation).

During observation of gonadal development the animals were handled with great care by a small rectangular scoop net as stress due to handling inhibits maturation of gonads in captivity. Care was taken to see the functioning of aerators, biological filters and removal of unused food material which may leads to deterioration of water quality and resorption of developing ovaries. The maturation tank was covered with a hard cardboard and no artificial illumination was provided as the intensity of light seems to affect the maturation process. Otherwise strong light may be a source of stress to the prawns, especially the non-burrowing wandering animal like P. indicus.

5. EYESTALK ABLATION

For the experiment healthy, older animals of 130 ± 10 mm body length and 20 ± 2 gm body weight with complete appendages were selected which were more responsive to maturation than younger ones. The maturation tank was thoroughly washed and filled with filtered clean sea water of salinity 33.7‰. In the tank 4 females and 2 males were kept. The females

were subjected to eyestalk ablation using an electrocautery apparatus, heated with 6 volts current **[Plate-1c]**. Cauterisation prevented bleeding and there was practically no mortality due to eyestalk ablation. The males were not eye ablated. For unilateral eyestalk ablation experiment, only one eye of each female prawn was removed **[Plate-2a]**, whereas for bilateral eyestalk ablation experiment, both eyes were removed **[Plate-2b]**. The ablated eyes were separately taken for electrophoretic studies. In another tank 3 females and 2 males were kept as control (without eyestalk ablation) providing same environmental condition as that of experiment. The main aim of keeping the males with females were to provide a suitable breeding environment as happening in open sea. The prawns meant for control were sacrified periodically to see the protein patterns in selected tissues before eyestalk ablation. The eyestalk ablated (both unilateral and bilateral) prawns were sacrified after 7 days, 15 days and 30 days to see the protein patterns after eyestalk ablation in selected tissues.

DISC ELECTROPHORESIS METHOD

1. PRINCIPLE

Electrophoresis is a technique where separation and migration of soluble protein mixtures occur due to differences in their net charge and molecular size. A buffer of appropriate pH was used to prepare the supporting gel medium (Polyacrylamide gel) on which sample was placed and electrophoresis conducted. Proteins consists of aminoacids with electrically charged side chains. Thus all proteins have a net electric charge

depending on the relative proportions of aminoacids unless they are at their isoelectric point, the pH at which the net charge becomes zero. When a current is passed through the gel made in a suitable buffer pH, the different proteins migrate towards the pole of opposite charge at a rate which was proportional to the magnitude of their charge and thus gradually get separated. The separated protein bands pattern made in a visible form using appropriate protein stains is called a Zymogram/Electrophorogram. The proteins with their marginal structural differences can thus easily be distinguished and studied.

2. ELECTROPHORESIS PROCEDURE

The method followed here is a modified original Ornstein and Davis (1962) method called as discontinuous disc electrophoresis, where tank buffer and gel buffer were quite different from each other (Diety et al., 1972).

2.1. Apparatus used: The cylinderical perspex tanks manufactured by M.C.Dalal and Co, with facilities to run 12 tube gels at a time was used. A power pack manufactured by Biochem company (Model-LK 69 D) was used for supplying power. Hand homogenized tissues were centrifuged in the Sorvall-5B Refrigerated superspeed centrifuge unit. The gel tubes of 85 mm total length and a inner dia of 5 mm were used for setting the gels. Each gel tube was engraved with markings in upper edge. Two numbers of digital micropipettes with 25-40 /ul and 40-100 /ul capacity were used for applying a known quantity of sample **[Plate-2c]**.

2.2 Standardization: The objective of standardization of methodology to be adopted in the present study was to evolve a suitable standard electrophoresis procedure to obtain better separation and resolution of general protein mixtures contained in different tissues of Penaeus indicus. For the standardization of methodology several electrophoresis runs were carried out by taking different gel concentrations 10%(A) + 2%(B), 9%(A) + 2%(B) 7%(A) + 2%(B), 5%(A) + 2%(B)], different weights of tissue for homogenization (50 mg/1 ml DDW, 100 mg/1 ml DDW, 200 mg/1ml DDW), different sample quantities (40 /ul, 50 /ul, 75 /ul, 100 /ul), supply of different amount of power (30 MA, 40 MA, 50 MA) and different time periods of staining with AB and CBB stains.

2.3. Preparation of gel and buffer stock solutions: The polyacrylamide tube gels were prepared immediately before electrophoresis from a number of highly toxic synthetic chemicals by polymerization reaction. The Acrylamide monomer (A) CH_2=CHCONH$_2$ was copolymerized with a cross linking agent usually N, N^1 - methylene - Bisacrylamide (B) $CH_2(NHCOCH = CH_2)_2$ in the presence of a catalyst accelerater mixture. This mixture was mainly consists of N, N, N^1, N^1 - tetramethylenediamine (TEMED) and double strength Ammonium persulfate. The porosity of the gel was determined by the relative proportion of Acrylamide (A) monomer to cross linking agent Bisacrylamide (B) with double distilled water (DDW). For general protein study Tris-Glycine was used as tank buffer and Tris-Hcl as gel buffer.

2.3.1. Gel buffer (Tris Hcl, pH 8.9):

i) 48 ml of 1N Hcl acid

ii) 36.6 gm of Tris-buffer (Tri-hydroxy methyl aminomethane)

iii) 0.23 ml of N, N, N^1, N^1 - Tetramethylethelenediamine (TEMED)

All reagents were dissolved in DDW and volume made upto 100 ml. The pH of the solution was checked with battery operated pH meter. The pH was adjusted by adding either concentrated Hcl acid or concentrated NaOH solution as required.

2.3.2 Tank buffer (Tris-Glycine, pH 8.3):

i) 6 gm of 0.05M Tris-buffer

ii) 28.8 gm of 0.38M Glycine.

All reagents were dissolved in DDW (W/v), volume made upto 1 litre and stored at 4°C. Before use 60 ml of this stock solution was diluted to 600 ml by adding DDW (V/V).

2.3.3 Acrylamide (A) 40%:

40 gm of Acrylamide (A) was dissolved in 100 ml of DDW (W/v).

2.3.4. Bisacrylamide (B) 2.1%:

2.1 gm of Bisacrylamide (B) was dissolved in 100 ml of DDW(W/v)

2.3.5. Double strength Ammonium persulfate:

280 mg of Ammonium persulfate was dissolved in 100 ml DDW (W/v).

2.3.6. Marker Dye:

Bromophenol blue (BPB) 0.1% was prepared by dissolving 100 mg of BPB in 100 ml DDW (W/v). Sucroge 40% was prepared by dissolving 40 gms of sucrose in 100 ml DDW (W/v). 1 part of 0.1% BPB was mixed with same part of 40% sucrose solution. Then the whole mixture was diluted with DDW in 1:1 ratio (V/V).

2.3.7. Stains:

i) Amido Black (AB) 0.1%: 1 gm of AB was dissolved in 1 litre of DDW (W/v).

ii) Coomossie Brilliant Blue (CBB) 0.2%: 2 gm of CBB was dissolved in 1 litre of Methanol, DDW and Acetic acid prepared in 5:4:1 proportion.

2.3.8. Destain:

Acetic acid 7%: 70 ml of glacial acetic acid was dissolved in DDW and volume made upto 1 litre by adding DDW (V/V).

2.3.9. Fixative:

10% Trichloro acetic acid (TCA): 100 gm of TCA was dissolved in 1 litre of DDW.

2.4. Preparation of tissue extract: The animals were collected from the experimental tanks, their total length (from tip of rostrum to tip of telson excluding last spine) and weights were taken. For collecting the hemolymph (HM) a glass syringe of 0.5 ml capacity with No.24 needle was used.

Prior to the collection of HM, the carapace and adjacent areas of the prawns were thoroughly blotted with absorbent paper to remove excess water attached to the body surface. The HM was directly drawn from the heart by keeping the animal in a bending position. The HM was poured into the preweighted sample vials and kept inside the freezer. Then the other tissues like Eye (E), Hepatopancreas (HP), Gonad (G), Nerve (N), Antenna base (A) Body muscle (M) were removed, weighed and homogenised with the DDW @ 100 mg/1 ml by hand homogenizer. The whole nervous system was taken for preparation of nerve tissue extract **[Plate-3c]**. The homogenized tissues were centrifuged for 20 minutes in 10000 rpm at below 10°C.

2.5. Setting of gels: The stock solutions like Acrylamide (A), Bisacrylamide (B), Tris-Hcl and Ammonium persulfate were brought to room temperature before preparation of the gel. The clean gel tubes were carefully inserted into the rubber grommet of gel stand. The above solutions were thoroughly mixed according to their respective combination **[Table-1]**. Care was taken to avoid trapping of air which otherwise slowly rise and form bubbles in the gel and inhibit polymerization. The mixed gel solution was carefully added with help of a glass syringe to a height of 75 mm. Immediately water was layered carefully above the gel by a plastic syringe without disturbing the gel bed. The function of the water layer was to overlay the gel solution so that it was polymerized with a flat surface devoid of meniscus distortion. The gels were set in about 20-30 minutes in room temperature. After polymerization the water layer was decanted.

2.6. Application of sample and marker dye: For applications of samples the clear supernatant portion of the centrifuged fluid was taken. Samples of required quantity (50 /ul, 75 /ul, 100 /ul) were applied by means of digital micropipette over the gel. Then 20 /ul of marker dye was applied over the sample and properly mixed. The main function of sucrose was to keep the sample separate from the buffer and to ensure a uniform flow of current. The remaining part of the gel tube was filled with tank buffer.

2.7. Setting of disc unit: The gel tubes were then removed from gel stand and inserted into the rubber grommets of the upper tank of disc electrophoresis unit from the lower side in such a way that the upper end of gel tubes were just above the lower surface of the upper tank. First the lower buffer tank was filled with tank buffer (Tris-glycine) upto the mark and then attached with anode (+ve) wire of power pack. Then the upper tank was fixed on to the lower tank of the unit and attached with cathode (-ve) wire of power pack. The power pack was switched on and initially adjusted to supply 12 Milli Ampere (MA) for 10 minutes, and then 36 MA for another 2 hours 20 minutes. The current was regulated for uniform flow of 3 MA/gel tube.

2.8 Removal of gels: The power pack was switched off when the marker dye migrated to the lower end of the gel. The tubes were removed from the grommets and placed in a petridish. The total length of gels and marker dye length were measured. The gels were then removed from the gel tubes by forcing a jet of water between the gel and the inner wall of the gel tubes using a syringe without damaging the gels.

2.9. Staining and destaining: As most proteins are colourless, the separated protein bands were made visible only after an appropriate staining. The general proteins studied here were stained with Amido Black (AB) or Coomassie Brilliant Blue (CBB). Before staining the gels were fixed with 10% TCA for 10 minutes. The stainaing period was only 10 minutes. The destaining procedures were carried out with 7% Acetic acid for 2-3 days.

2.10. Zymogram and photography: Some degree of distortion of the tube gel surface was suspected in certain gel tubes. Hence the zymogram was drawn by taking the marker dye as the base to obtain a uniform rate of migration inspite of such minor distortion. For zymogram and photography gels were kept in a clear test tube and filled it with DDW and stoppered the tube to avoid air bubbles.

RESULTS AND DISCUSSION

RESULTS

1. STANDARDISATION OF METHODOLOGY

The results presented here were obtained after necessary standardisation of various aspects of disc gel electrophoresis method described by Subhashini and Rabindranath (1981), Ponniah (1983). The basis for the standardisation or improvement of the methodology was with special reference to percentage of gel composition that separated and resolved maximum number of general protein bands. Among different percentage of gel composition tried, 10% (A) + 2% (A) polyacrylamide gel was found to produce a satisfactory separation and resolution of general proteins in all the tissues of P. indicus tested in the present study **[Plate-5-8]**. The standardisation was also based on the comparison of different quantities of tissue homoginate samples for producing better results. In this respect 100 mg/1 ml DDW was found to be a standard volume for sample preparation and 100 /ul for sample application in all experiment. For purpose of staining general protein 0.1% AB and 0.2% CBB stains were tried. A comparison of the results obtained by these two stains produced relatively different patterns in different tissues tested. On the basis of clarity and staining intensity of different major and minor bands in different tissues tested, AB was found to be a better stain for general proteins of P. indicus. As minimum period for staining all the general protein bands was necessary, different

staining periods were tried. A Standard staining time of 10 minutes produced all the major and minor protein bands in different tissues. For purpose of destaining 7% Acetic acid was found to produce easy destaining within 2-3 days **[Plate-4a,b,c].**

2. EFFECTS OF EYESTALK ABLATION

To get an insight into the effects of eyestalk ablation on the electrophoretic patterns of general proteins obtained from P. indicus the results are detailed and described under 3 headings namely:

1. Patterns before eyestalk ablation
2. Patterns after a certain period of unilateral eyestalk ablation
3. Patterns after a certain period of bilateral eyestalk ablation **[Table 3-5, Fig.1-7].**

The table 6 on the other hand shows the protein patterns that were present in the brood female collected from the open sea and spawned specimen after unilateral eyestalk ablation. The protein patterns present in the control specimen tested after selected period of time are shown in the brackets of table-7.

To demonstrate the effects of eyestalk ablation a number of likely target tissues like Hemolymph (HM), Ovary (G), Nervous system (N), Hepato-pancreas (HP), Antenna base (A) and Body muscle (M) etc. were selected as source of test samples. The actual effects of eyestalk ablation on the

general protein patterns in different target tissues mentioned above were demonstrated here mainly by two methods, considering:

1. The total number of protein bands having a range of visually estimable colour staining intensity of each band [column 3 - 11 of **Tables 3-6]**.

2. Details of distance migrated by individual protein bands (electrophoretic mobility) with reference to the mobility of BPB marker dye and estimable colour intensity of each band **[Table 8-26].** There is a special reason for taking the BPB marker dye as the base for measuring the migration distance of protein bands instead of the usual gel top where the samples were introduced. Because there was marked variation in the distance of BPB marker dye moved in different gel tubes under single experiment with uniform electrophoretic conditions like percentage of gel composition, pH, current applied etc. This is presumably due to certain artefacts that may occur during the gel casting process, causing slight variatio in the length and strength of the gel. This was evident in the result shown in table-3 where the range of mmarker dye movement was 68-75 mm under the same experiment.

2.1. Protein patterns before eyestalk ablation: The basic patterns of general protein in the 7 target tissues including the Eye (E) before eyestalk ablation indicated that all tissues possessed a tissue specific protein patterns either in terms of differences in the total number of bands or

the colour intensity of each specific band or electrophoretic mobility of a particular band **[Table-3,8-10; Fig.1; Plate-5a,b,c].**

A comparison on the basis of number of major and minor protein bands present in each target tissue tested here showed a significant range in the number. The maximum number of protein bands 16 in HP and least number of 4 bands in N tissue indicated such a range. A comparison of degree of staining intensity of protein bands of different target tissues showed tissue specific differences **[Table-7].** It is also seen that the above tissue specific protein patterns in each individual target tissue are retained throughout the experimental period, irrespective of periodical test or in spite of positive effects of eyestalk ablation on the pattern of this proteins **[Table-3-6,9].**

2.2. Protein patterns after unilateral eyestalk ablation: A comparison of general protein patterns obtained in different tissues after 7 days of unilateral eyestalk ablation**[Table-11,12; Fig.2; Plate-6a]** with that of unablated **[Table-3,8-10; Fig.1; Plate-5a,b,c]**, revealed a significant variation. On 7th day the number of protein bands in all the tissues of ablated specimen of P. indicus was found increased considerably. The significant increase in the number of protein bands in G (5 in unablated and 17 in ablated) and in HO (16 in unablated and 25 in ablated) is a positive effect of eyestalk ablation. Equally important was the increase of protein bands of N tissue from 4 in unablated to 7 in ablated on the 7th day. The number of bands increased in HM and E was 5 to 7 and 7 to 8 in the tissues of unablated

and ablated condition respectively. A comparison of colour intensity of different protein bands in different tissues of specimen before eyestalk ablation and after unilateral eyestalk ablation on 7th day also indicates variations **[Table-3,4]**. The effects of unilateral eyestalk ablation on the protein patterns in terms of their total number also appear to be considerably different particularly in certain tissues on 15th day compared to 7th day as well as zero day (control), where as the 17 number of protein bands of G also remained unchanged on the 15th day **[Table-7]**. The HP with 25 bands on 7th day showed only 13 bands on 15th day **[Table-13]**. The number of bands in HM, E and N also showed a reduction towards the 15th day of unilateral eyestalk ablation **[Table-13,14; Fig.3; Plate-6c]**. Besides, a comparison of the number of protein bands obtained on 15th day with that of zero day (control) again showed a decrease in all tissues except in the G where the increased number 17 registered on 7th day remained steady on 15th day also **[Table-7]**.

The effects of unilateral eyestalk ablation was clearly noticeable even on 30th day. The number of protein bands present in HP (13) and G (17) on 15th day considerably got reduced to 7 and 12 respectively on 30th day, where as such reduction was more significant in the case of HP, compared to its pattern on 7th day, the reduction being from 25 to 7 **[Table-15; Fig.4; Plate-7a]**.

Another important aspects of the effect of unilateral eyestalk ablation on the general protein of P. indicus was with reference to the

distance migrated by each protein band and its staining intensity **[Table-11-17; Fig.2-4]**.

A comparison of this parameter in the specimen before ablation and on a particular period after unilateral eyestalk ablation showed considerable differences **[Table-7]**. For example, a certain characteristic electrophoretic mobility of protein bands and staining intensity possessed by each band of HP before eyestalk ablation was detailed in the table-8 and figure-1 got altered on 7th, 15th and 30th days of unilateral eyestalk ablation for almost all bands **[Table-11-17; Fig.2-4]**. Similar changes were also noticeable, though at different degrees, in the case of protein patterns produced in all other tissues tested here. This secondary aspect of variations clearly induced by the effects of unilateral eyestalk ablation are too complex to be sorted out in more precise patterns because of the appearance or disappearance of new or earlier bands and increase or decrease of staining intensity of some bands or changes in electrophoretic mobility of some bands during the particular period of the experiment.

2.3. Protein patterns after bilateral eyestalk ablation: A comparison of general protein patterns obtained in different tissues before eyestalk ablation with that of bilateral eyestalk ablation on 7th, 15th and 30th days again showed interestingly different form of results **[Table-5,7,18-24; Fig.5-7; Plate-6b, 7b,c, 8]**. For example, on 7th day of bilateral eyestalk ablation experiment,the tissues like HP and G showed a significantly negative response compared to significantly positive response by these two tissues to the unilateral eyestalk ablation. Because the 16 number of HP bands on zero

day (control) got reduced to 7 on 7th day of bilateral eyestalk ablation, whereas 16 number of HP bands on zero day (control) got increased to 25 bands on 7th day of unilateral eyestalk ablation. Similarly 7 number protein bands of G on zero day (control) got reduced to 6 bands on 7th day of bilateral eyestalk ablation experiment. On the other hand, 5 number of HM protein bands on zero day got increased to 8 on 7th day of bilateral eyestalk ablation experiment which is comparable to that of unilateral eyestalk ablation experiment for the same period **[Table-7]**.

An equally interesting result was obtained in the case of N tissue. The number of protein bands which was 4 on zero day remained unchanged on 7th day of bilateral eyestalk ablation experiment, whereas the 4 number of N bands on zero day got increased to 7 on 7th day during unilateral eyestalk ablation experiment.

The general protein pattern of all the tissues tested on 15th day under bilateral eyestalk ablation experiment was dramatically different from the patterns described in either of these experiments. Because the 6 number of G on 7th day got increased by more than 100% (13 bands) on 15th day of bilateral eyestalk ablation experiment, whereas 17 number of G bands obtained on 7th day remained unchanged on 15th day under unilateral eyestalk ablation experiment. In the case of N, the number of 4 bands on 7th day got increased to 7 on 15th day of bilateral eyestalk ablation experiment, whereas the 7 number of N protein bands on 7th day got significantly reduced to 2 on 15th day under unilateral eyestalk ablation

experiment. The only comparable patterns produced due to the effects of unilateral as well as bilateral eyestalk ablation experiment was similar response of HM. Because 7 number of HM protein bands on 7th day got reduced to 4 bands on 15th day under unilateral eyestalk ablation experiment and 8 bands on 7th day also got reduced to 4 on 15th day under bilateral eyestalk ablation experiment **[Table-7]**.

To get further insight into the effects of unilateral eyestalk ablation before spawning and after spawning in the general protein patterns of different tissues of P. indicus, a specimen which was spawned on 5th day under unilateral eyestalk ablation was analysed on 6th day. The details of the results obtained are shown in the **[Table-6]**. A comparison of the protein pattern of different tissues on 7th day of unilateral eyestalk ablation experiment of the present study and that of the spawned specimen on 6th day of unilateral eyestalk ablation showed a significantly contrasting patterns in almost all tissues. For example, 25 number of protein bands in HP on 7th day of unilateral eyestalk ablation of the present study got reduced to the extend of 7 bands in the spawned specimen. Similarly, 17 number of protein bands in G, 7 number in HM, 8 number in E and 7 number in N got reduced to 9, 3, 5 and 4 respectively **[Table-7]**. Again, the protein patterns of spawned specimen showed differences compared to that of zero day. All tissues except Ovary of spawned specimen had considerably lesser number of protein bands compared to that of unablated zero day specimen. Particularly significant difference was noticed in the HP tissue which had 16 bands during zero day and 25 bands on 7th day and 13 bands on 15th

day got reduced to merely 7 bands in the spawned specimen **[Table-6]**. However the number of G protein bands were still relatively higher than that of zero day, it being 7 and 9 in respective days. On the other hand the number of G protein bands of spawned specimen was significantly lesser than that of either 7th day of 15th day, it being, 17 and 9 respectively. But the number of protein bands in N tissue on zero day and in spawned specimen was similar, it being 4 in both cases. However, it is interesting to note almost comparable pattern of proteins in all the tissues except in HP of spawned specimen and the pattern obtained on 30th day of unilateral eye stalk ablation **[Table-7]**. Again the protein patterns of spawned specimen was considerably different from that of late maturing stage specimen collected from wild. Significant reduction in the total number of protein bands was noticed in the HM and HP tissues, it being 10 and 14 in late maturing wild specimen, 3 and 7 in the spawned specimen respectively **[Table-6,7,25; Fig.8; Plate-7a]**.

DISCUSSION

In presenting the results of the effects of eyestalk ablation on the general proteins of Penaeus indicus studied here, it is clearly understood that the effects of eyestalk ablation were neither attempted literally from the same specimen nor possible to do so due to certain inherent constraints in the present experiment. Because the first specimen used for studying the general protein patterns of different tissues before eyestalk ablation

was sacrificed while taking its tissues for experiment. Therefore, to study the effects of eyestalk ablation of 7th, 15th and 30th days of ablation, different specimens were used, as at the end of each experimental days the involved specimens were naturally sacrificed for testing their tissues. However, the protein patterns of eye (E) before and after ablation of the corresponding periods was obtained from the same individual as a pair of eyestalk was available for such experiment. To reduce the above inherent constraints, the specimens were selected having a closely comparable length, weight and maturity stages so as to produce a reasonably interpretable scientific data. The scientific basis of the present experiment is also assured from the pattern of results obtained. The very high range of differences in the results shown suggests that the results obtained were not due to natural individual variations but as a consequence of the effects of eyestalk ablation itself. The above reasonable assumption is well supported by the experimental results obtained as a result of the effect of eyestalk ablation on various biological process of many other crustaceans (Adiyodi and Adiyodi, 1970).

Since the classical eyestalk ablation experiment of Panouse (1943) the role of crustacean eyestalk hormones in certain biological process, particularly, molting, development and maturation of gonad is well established This particular knowledge is currently applied for achieving certain objectives in the corresponding fields of research (Aoto and Nishida, 1956; Demeusy, 1962; Rangnekar and Deshmukh, 1968; Adiyodi and Adiyodi, 1970; Kamiguychi, 1971; Muthu and Laxminarayanan, 1979, 1982; Muthu et al, 1980, 1984 , 1986; Bomirski and Klek, 1974; Nagabhushanam and Diwan, 1974; Klek and

Bomirski, 1975; Laubier, 1975; Arnstein and Beard, 1975; Aquacop, 1975, 1982; Alikunhi et al., 1975; Webb, 1977; Rajyalakshmi et al., 1987c).

The inhibiting or promoting role of eyestalk in somatic growth and maturation of gonad was further explained by the antagonistic or synergestic action of hormones like OIH and MIH produced by the eyestalk with other hormones like OSH and MSH produced by other neuroendocrine centres of crustacea (Carlisle and Knowles, 1959; Waterman, 1969; Adiyodi and Adiyodi, 1970; Kulkarni and Nagabhushanam, 1979, 1980; Nagabhushanam and Kulkarni, 1982).

It is also known that there is a host of biochemical processes or physiological mechanism that follow the eyestalk ablation leading to the actual incidence of gonadal maturation or molting **[Plate 3a,3b]**. These processes occurring during the periods in between the eystalk ablation and the consequent molting or maturation, as the situation demands, involves additional or extra production of many known organic substances like proteins, lipids and free sugars etc., in specialised primary target tissues like HP, HM, M, G and their mobilisation from such extra ovarian production centres and their utilisation in the final target tissues like cells of ovary for its maturation process (Adiyodi 1968, 1968a, 1969a, 1978; Adiyodi and Adiyodi, 1970a, 1972 and Quackenbush, 1989).

Measurements of these organic substances like lipids, free sugars, and proteins produced in different target tissues like HP, HM and G etc, as a result of eyestalk ablation in selected crustaceans have already revealed

that their quantity mainly in terms of total concentration varies significantly before and after eyestalk ablation and also at different periods after ablation. General trend of such variation shows dramatic increase in these organic substances in certain tissues like HP and G and later significant decrease in the same tissues as they were mobilized, and utilised for the purpose for which they were produced namely for gonadal maturation or molting (Adiyodi, 1969a; Adiyodi and Adiyodi, 1970, 1972; Barlow and Ridgway, 1969; Goodwin, 1951; McWhinne and Mohrherr, 1970; Kulkarni and Nagabhushanam, 1979; Telford, 1968; Skinner, 1965, 1966; Kurup and Sheer, 1966 and Yamaoka and Raghavaiah, 1977, Quackenbush, 1986).

The results of present study on the effects of unilateral eyestalk ablation conducted in P. indicus corroborate the above reports of positive effect of similar studies conducted in other crustaceans. Thus the total number of protein bands counted as electrophoretic bands in the ovary before eyestalk ablation in P. indicus was only 7 which was found increased to 17 bands giving 143% increase after 7 days of eyestalk ablation. Such significant increase in the ovary proteins was also reflected in the increased size of the ovary on 7th day **[Plate-3b].** The increase in the protein bands in the case of N tissue was from 4 to 7 at the end of the 7th day of unilateral eyestalk ablation giving 75% increase over the zero day **[Table-9; Fig.1; Plate-6a].** The total number of HP protein bands was only 16 before ablation whereas it was found increased to 25 on 7th day of unilateral eyestalk ablation, giving 56% increase **[Table 3,4-8; Plate-5,6a].** These

three above results indicated a significant effect of unilateral eyestalk ablation on the general protein patterns in P. indicus. Though other tissues like HM, E showed relatively less increase in total number of general protein bands, the positive effect of unilateral eyestalk ablation in all these tissues also evidently demonstrated. This dramatic positive responses of G, N and HP of P. indicus to the unilateral eyestalk ablation is a clear demonstration of the inhibiting role naturally played by the eyestalk in gonadal maturation during unfavourable natural brackishwater conditions. Thus the actual inhibiting role of eyestalk appears to create a negative response in the target tissues like G, N and HP which act as production centres of protein or storage sites of such extra proteins essentially required for development and maturation of gonadal cells. Whether these protein bands electrophoretically detected and demonstrated in the G, N and HP of P. indicus studied here are precursors of egg yolk proteins or lipid carrier proteins are not verified as in the case of other penaeid prawn like P. vannamei (Quackenbush, 1989).

The other tissues responded positively on the 7th day of ablation but to a lesser extend than by G, N and HP were HM and the other eyestalk of P. indicus subjected to unilateral eyestalk ablation **[Table-4]**.

Thus the most significant effect of the present unilateral eyestalk ablation in P. indicus was the 143% increase in the number of protein bands of ovary (G) **[Table 4,12,14,16]**. Such highly significant sudden response of ovary (G) can be expected on sudden removal of the eyestalk which

had been inhibiting such positive response until the very moment of ablation Such varying response in terms of total concentrations of protein, free sugars and lipid in different tissues under natural and induced maturation conditions was also reported in P. indicus by earlier studies (Asokan, 1983).

Though, vitellogenin, a precursor of egg yolk protein, is synthesized in large scale in extra ovarian tissues like liver in vertebrates and fat bodies in insects, the extra ovarian tissues was suspected as the major site of vitellogenin production in crustaceans. However, electron microscopy and autoradiography studies in decapod crustaceans showed that ovary is the site of vitellogenin production (Adiyoi and Adiyodi, 1968; Beams and Kessel, 1963, Ganion and Kessel, 1972; Yano and Chinzei, 1987). When HP and ovary of P. japonicus and P. vennamei were tested, ovary was found to be the site of egg yolk protein synthesis (Yano and Chinzei, 1987; Quackenbush, 1986). Hence the highly positive response of ovary in P. indicus to the eyestalk ablation observed here may be considered as another example of ovary as the major site of egg yolk protein synthesis, assuming that the protein bands detected in the present study are egg yolk protein or its precursors.

The nervous system tissue (N) and HP were the other tissues of P. indicus that showed significant effects of eyestalk ablation registering 75% and 56% increase in the number of protein bands respectively. The N tissue tested in the present experiment actually consisted of the whole nervous system except that of eyestalk **[Plate-3c]**. The increase

of 75% in the number of N tissue protein bands as an effect of eyestalk ablation is very interesting. It shows that eyestalk endocrine factors of P. indicus have a wide range of target tissues under its direct or indirect inhibitory influence. Though susceptibility of crustacean central nervous (CNS) to hormonal priming action is suspected (Anil Kumar and Adiyodi, 1978), this is probably the first report of active response by the CNS to eyestalk ablation in terms of number of electrophoretic protein bands in crustaceans particularly in P. indicus.

The production of extra protein bands in N tissue after eyestalk ablation in P. indicus is again more interesting in view of the experimental report of induction of acceleration of ovarian growth by N tissue extract (brain and thoracic ganglia) into an eyestalk ablated as well as normal P. hardwickii (Nagabhushanam and Kulkarni, 1982). In the case of P. hardwickii the experiment indicated that brain and thoracic ganglia contains an ovarian growth inducing factor. However the source of the brain and thoracic ganglia extract was not mentioned as to the effect whether it was taken from a mature specimen or an eyestalk ablated or unablated specimen etc. To study the relationship between the extra protein bands produced in the N tissue of P. indicus subjected to unilateral ablation of the present experiment and the factors contained in the brain and thoracic ganglia of P. hardwickii that induced ovarian growth, a series of detailed comparative analytical experiments involving both species are necessary. However the present experiment clearly revealed the profound effects of unilateral eyestalk ablation on nervous system (N) in P. indicus.

The third tissue of P. indicus that showed a significant positive response to eyestalk ablation was HP. On the 7th day of unilateral eyestalk ablation the number of general protein bands were found increased by 56% **[Table-11,12; Fig.2; Plate-6a].** The internal organ described as the HP in crustacean is a vital organ of physiological importance. It may be functionally comparable to the liver of vertebrates. It is an important storage organ of organic/inorganic resources like proteins, lipids and free sugars etc, required for many physiological functions including molting and reproduction. Certain aspects of the relationship between the HP function and eyestalk have been well established particularly on the basis of eyestalk ablation and its effects on the quantity and quality of organic resources of HP in decapods (Fingerman, 1987; Lockwood, 1967; Adiyodi and Adiyodi, 1970; 1970a; 1972; paulus and Laufer, 1987; Quackenbush, 1986).

These studies demonstrated that the quantities of phospholipids, proteins and free sugars etc. are increased and stored during intermolt stage and later decreased as these resources are utilized during molting/reproduction (Adiyodi and Adiyodi, 1970, 1970a, 1972, 1972a).

The positive effects of eyestalk ablation on the electrophoretic patterns of proteins in the HP of the juvenile crab P. hydrodromous showed a definite variation in the pattern on different days after ablation. In the above report the reversal of protein pattern variation to the normal pattern was achieved by injecting eyestalk extract into a destalked crab, thus clearly demonstrated that the observed variation was due to the eyestalk

removal only (Adiyodi and Adiyodi, 1982). Hence the present observation of 56% increase in HP protein bands in the destalked P. indicus on the 7th day of ablation can be reasonably be correlated to the direct positive effects of eyestalk ablation.

The HM of P. indicus tested in the present study also showed 40% increase in the number of electrophoretic protein bands as a result of the effects of eyestalk ablation **[Table 2,3 and 10]**. Increase in the HM protein concentration and electrophoretic bands particularly the female specific protein (FSP) were found increased maximum in normal unablated lobster H. americanus specimen just prior to oviposition (Byard and Aiken, 1984), and occurrence of higher concentration of HM protein was also observed in many other crustaceans as effects of either normal vitellogenesis processes or due to effects of eyestalk ablation (Adiyodi and Adiyodi, 1970; Quackenbush, 19886). These reports also suggest that seasonal variation in the HM proteins normally occur as a normal response to the current deominant physiological processes such as vitellogenesis or molting etc. Similar effects can be induced by the removal of the eyestalk that has been well established as the seat of endocrine factors controlling such normal physiological phenomena existing at a particular state of somatic growth or gonadal maturation as the case may be. There is no clear understanding as to the source of synthesis of these extra proteins appearing in the HM during vitellogenesis. Hemoloymph may be just a medium for transporting such proteins synthesized elsewhere.

The general agreement is that these proteins particularly the FSP appearing at the time of vitellogenesis may have been produced elsewhere but being carried through the HM to target site of oocytes (Adiyodi, 1985; Beard and Aiken, 1984; Quackenbush, 1989). Whether the additional proteins appeared in the HM of P. indicus studied here on the 7th day of ablation wwere synthesized there itself or were in transit or whether the protein was FSP/vitellogenin can not be inferred from the present investigation of a different nature.

The pattern of the results of the present study on the effects of unilateral eyestalk ablation in P. indicus discussed above was found again considerably varying on 15th and 30th days of ablation. Such variations were also reflected particularly with reference to the previous pattern existed, viz; on zero day, 7th day, respectively[Table-7].

However it is interesting to note that the 17 number of bands in ovary present on 7th day found unchanged on 15th day under unilateral eyestalk ablation, whereas it got reduced to 12 on 30th day, which still considerably higher than 7 bands existed on zero day. Similar tendency of reduction in the total number of protein bands on day 15th and 30th was noticed in other tissues like HM, HP, E and N particularly compared to the pattern obtained on 7th day in all tissues but only in certain cases compared to the patterns on 15th day. The occurrence of only 5 number protein bands in HM on 30th day under unilateral eyestalk ablation suggests the complete disappearance of the effects of unilateral eyestalk ablation,

because that was the normal pattern existed on zero day **[Table 7].**

It suggests that after particular period of unilateral eyestalk ablation the tendency of the tissue protein pattern is to return towards the original pattern even in the absence of one eyestalk. This can be due to a normal built in homostatic mechanism that becomes active and dominate during normal cyclic process of molting and reproduction. The considerable differences that existed between the pattern of general proteins in different tissues of a specimen induced to spawn on 5th day of unilateral eyestalk ablation and that of the specimens subjected to unilateral eyestalk ablation of the present investigation **[Table-7]** were naturally expected.

Though the tissue protein patterns of spawned specimen on 6th day of ablation showed considerably different pattern from that of either zero day, 7th day or 15th day the pattern of spawned specimen appears to be closely resembling to that 30th day of unilateral eyestalk ablation of the present experiment **[Table-25,26; Fig.8].** It suggests that the positive effect of eyestalk ablation disappears after certain periods irrespective of the specimen reached full maturation or attained spawning or not. This is due to the cyclic process of vitellogenesis, controlled by endocrine hormones in natural situation.

Another basic reason may be that the tissue specificity of general protein patterns inherited and controlled through species specific gene mechanism remains undisturbed in spite of a general changes brought about

by the eyestalk ablation in the number of bands or variation in the staining intensity.

A comparison of general protein pattern in different tissues before eyestalk ablation with that of bilateral eyestalk ablation on 7th, 15th and 30th days again showed an entirely different pattern compared to that of unilateral eyestalk ablation, particularly in the case of G, HP and N **[Table-7]**. The total number of protein bands on 7th day of bilateral ablation in the tissues like HP and ovary reflex a significantly negative response compared to significantly positive response by these same tissues to the unilateral eyestalk ablation during the same period. Because the HP with 16 bands on zero day of bilateral eyestalk ablation got reduced to 7 on 7th day and remained with 8 bands on 15th and 30th days. The G with 7 bands on zero day though reduced to only 6, increased to 13 on 15th day. The observation of 6 number of G bands on 7th day increasing to 13 on 15th day suggests a positive effect of bilateral eyestalk ablation after a longer period than that of unilateral eyestalk ablation **[Table-18; 22; Fig.5,6; Plate-7,8]**. In the case of N tissue it is interesting to note a comparable increase from 4 number bands on zero day to 7 on 15th day again suggesting a longer period to produce a positive effect on these tissues compared to the positive effects of unilateral eyestalk ablation for the same period. It may also suggest that the factors influenced the G tissue and N tissue are the same, because the positive effect was taken place only on 15th day in these two particular tissues. The delayed positive response of G, N and to a lesser extend by HP on 15th day but not earlier

as happened in the case of unilateral eyestalk ablation indicates that some kind of favourable endocrine mechanism has overtaken the negative response created by the removal of two eyestalks.

The only comparable effects observed under both unilateral and bilateral eyestalk ablation experiment was the positive response of HM tissue by increasing from 5 numbers to 7 on 7th day under unilateral eyestalk ablation and 8 on 7th day under bilateral eyestalk ablation and showing slight negative response by reducing the number to 4 on 15th day under unilateral eyestalk ablation and again the same 4 bands on 15th day under bilateral eyestalk ablation **[Table-7]**.

There are no reports on effects of bilateral eyestalk ablation on electrophoretically detected general protein in other crustaceans for comparison, except the general effects of bilateral eyestalk ablation on molting and gonad development (Caillouet, 1973; Duronslet et al., 1975; Aquacop, 1975; Wear and Santiago, 1976).

A close analysis of the present results of the effects of unilateral and bilateral eyestalk ablation reveals that the effects of either type of eyestalk ablation is not permanent in nature, that a physiological/biochemical change induced by eyestalk ablation leads to secondary changes as a results of the primary effect of eyestalk ablation. These results also suggests the complex role of antagonistic, synergestic and homostatic mechanism involved in producing the observed protein patterns in P. indicus.

Though the total number of protein bands of G tissue remained unchanged even on 15th day of unilateral eyestalk ablation, the quantitative patterns of protein in HM, HP, E and N were found considerably reduced with reference to the patterns of 7th day, but to a lesser extend compared to zero day **[Table-11-14]**. As the final effect of eyestalk ablation is expected to lead to building up of extra protein resources in ovary probably by mobilizing from other sources, the steady state of the number of bands in G even on the 15th day and meanwhile unsteady conditions of the number of protein bands in other tissues is natural. the above pattern of reduction of protein bands of HP, HM etc. agrees with such reductions in total concentration of proteins and other organic substances reported in P. indicus (Asokan, 1983).

The pattern of reduction showed 71% in the N tissue followed by 48% in HP tissue, 43% in HM and 25%in E tissue. The present report of unchanged G protein patterns in P. indicus even on 15th day of unilateral eyestalk ablation is comparable positively with comparable unchanged pattern observed on the 14th day of ablation of eyestalk in P. vennamei with special reference to immunprecipitated total protein. However it is interesting to note that the total protein in P. vannamei on 14th day remained stable until 30 day. Though the methodology of "Immunoprecipitation" adopted in the case of P. vannamei and that of the present study (Electrophoresis) was not comparable, the positive effect of eyestalk ablation in increasing either the quantity of total protein in P. vannamei or the total number of protein bands in P. indicus on 7th day of ablation and its stability until 15th day is well established beyond doubt.

The total number of protein bands in all the tissues tested in P. indicus on the 30th day of unilateral eyestalk ablation further got reduced considerably compared to 7th and 15th day **[Table-15-17; Fig.4; Plate-6c,7a].** The total number of ovary protein bands which remained unchanged on 15th day of ablation also showed a reduction of about 30% of its bands. However the highest reduction of 72% was recorded by the HP tissue, followed by N(50%), G (30%). these reductions were again of higher order compared to that of 7th day pattern. Inspite of considerable reductions in the number of protein bands on 30th day, ovary still showed more bands compared to that of 7th day. This is again expected because ovary is the final target tissue to reflect the total effects of eyestalk ablation, especially where gonadal cells progresses towards required size for vitellogeneis.

It is also important to note that the normal pattern of HM protein bands on zero day was returned on 30th day of ablation **[Table-7].** Probably there are no previous reports of results of the effects of eyestalk ablation on protein bands in crustacean on 15th day and beyond for comparison.

The effect of unilateral eyestalk ablation on the general proteins in tissues like G, N, HP & HM of Penaeus indicus was highly positive, particularly on 7th day of ablation, whereas, the effect was of lesser order on 15th and 30th days of ablation. On the other hand the effect of bilateral eyestalk ablation on the above same parameters was highly negative, particularly on 7th day of ablation but became reasonably positive on 15th

day of ablation particularly with reference proteins of ovary and nerve tissues. The protein patterns of all the above tissues except nervous tissue (N) of a unilateral ablated and spawned P. indicus were of different nature and order compared to the above experimental result.

FIG. 1. Zymograms of before eyestalk ablation.

FIG. . Zymograms of after 7 days of unilateral eyestalk ablation experiment.

FIG. 3. Zymograms of after 15 days of unilateral eyestalk ablation. experiment.

FIG. 4. Zymograms of after 30 days of Unilateral eyestalk ablation experiment.

FIG. 5. Zymograms of after 7 days of Bilateral eyestalk ablation experiment

FIG. 6. Zymograms of after 15 days of Bilateral eyestalk ablation experiment.

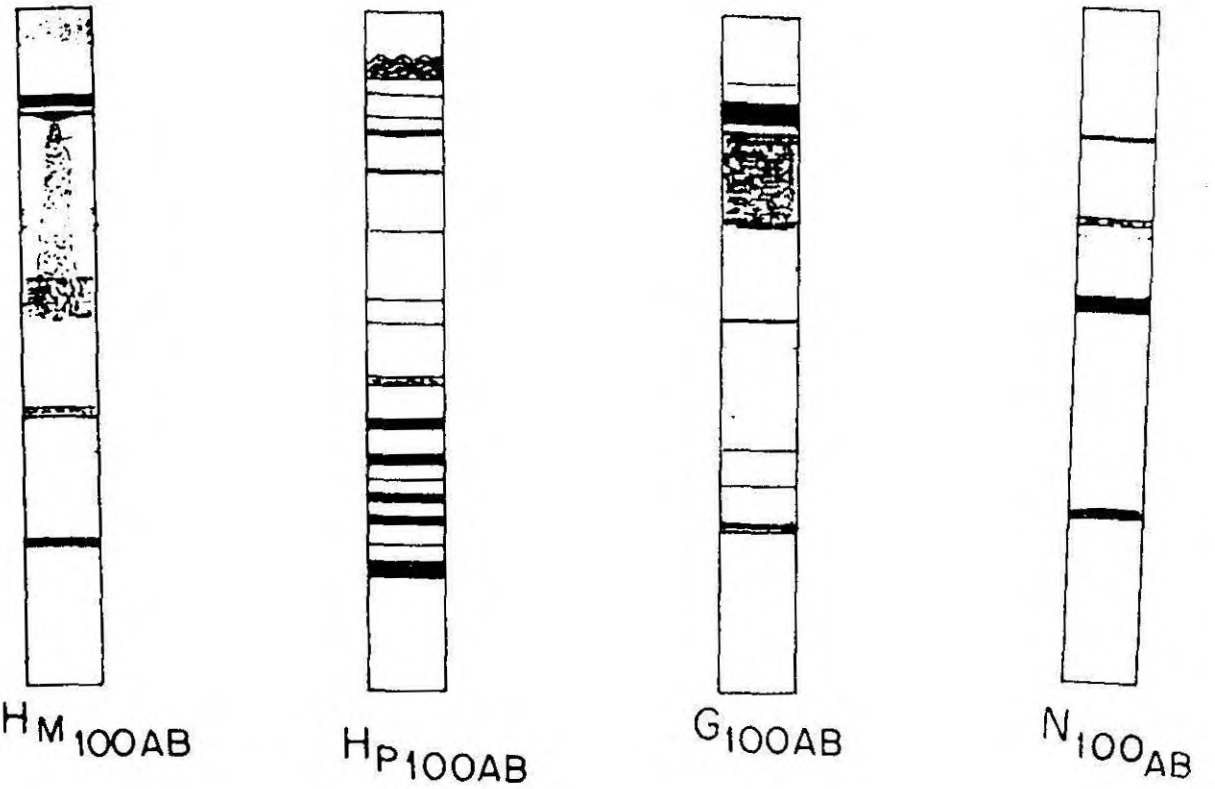
FIG. 7. Zymograms of after 30 days of Bilateral eyestalk ablation experiment.

FIG. 8. Zymograms of Wild specimen.

N.B.

M100 AB	Body Muscle of 100 /ul quantity in Amido black stain.
M100CBB	Body muscle of 100 /ul quantity in Coomossie Brilliant Blue.
E100CBB	Eye of 100 /ul quantity in Coomossie Brilliant blue.
A100AB	Antenna Base of 100 /ul quantity in Amido black stain
A100CBB	Antenna Base of 100 /ul quantity in Coomossie Brilliant blue.
HM100AB	Hemolymph of 100 /ul quantity in Amido black stain.
HM100CBB	Hemolymph of 100 /ul quantity in Coomossie Brilliant blue
HP100AB	Hepatopancreas of 100 /ul quantity in Amido black stain.
HP100CBB	Hepatopancreas of 100 /ul quantity in Coomossie Brilliant blue
G100AB	Gonad of 100 /ul quantity in Amido black stain.
G100CBB	Gonad of 100 /ul quantity in Coomossie Brilliant blue.
N100AB	Nerve of 100 /ul quantity in Amido black stain.
N100CBB	Nerve of 100 /ul quantity in Coomossie Brilliant blue
E100AB	Eye of 100 /ul quantity in Amido black stain.

FIG. 1


HM100AB
HP100AB
G100AB
N100AB

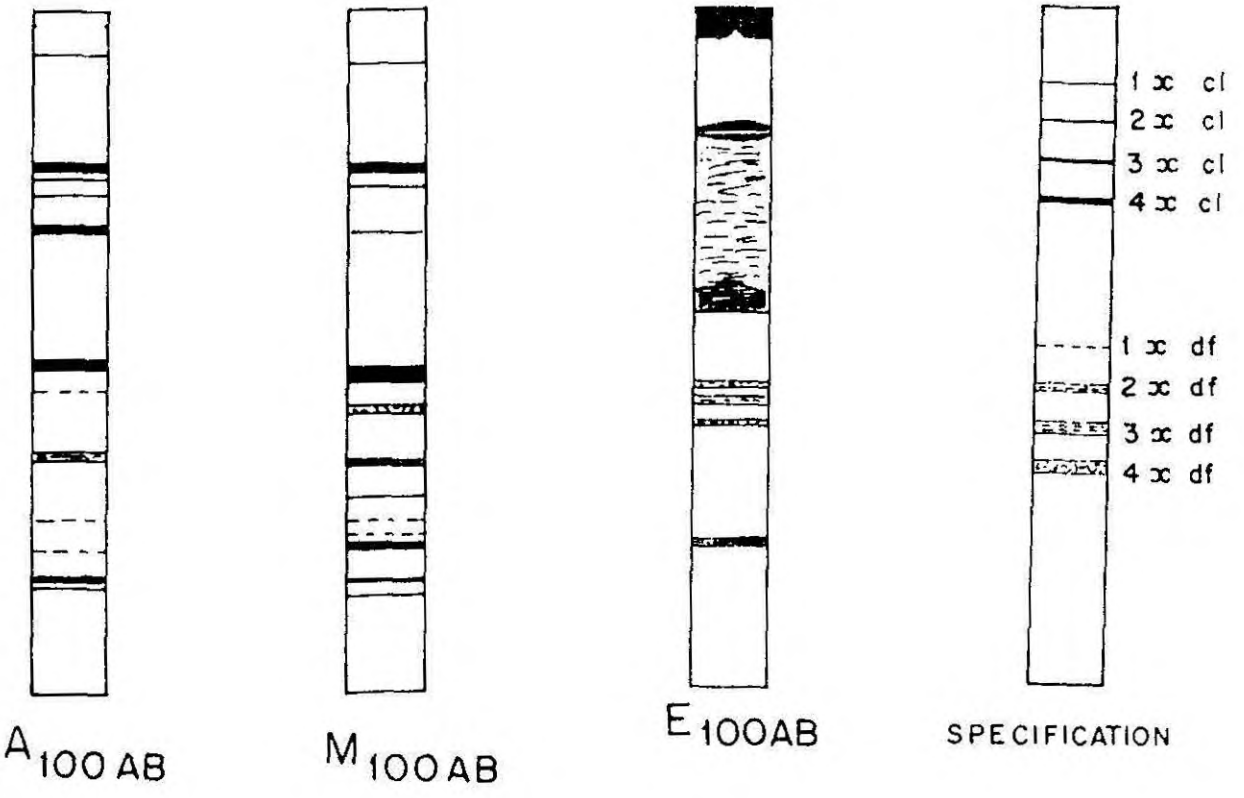

A100 AB
M100 AB
E100AB
1 x cl
2 x cl
3 x cl
4 x cl
1 x df
2 x df
3 x df
4 x df
SPECIFICATION

FIG. 2

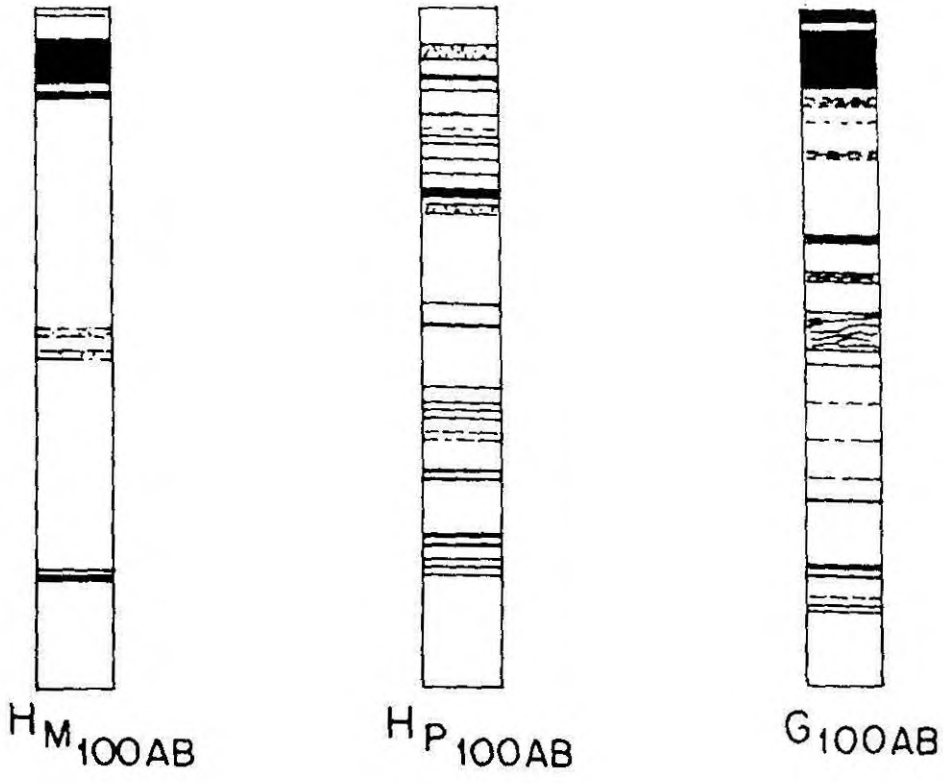


FIG. 3

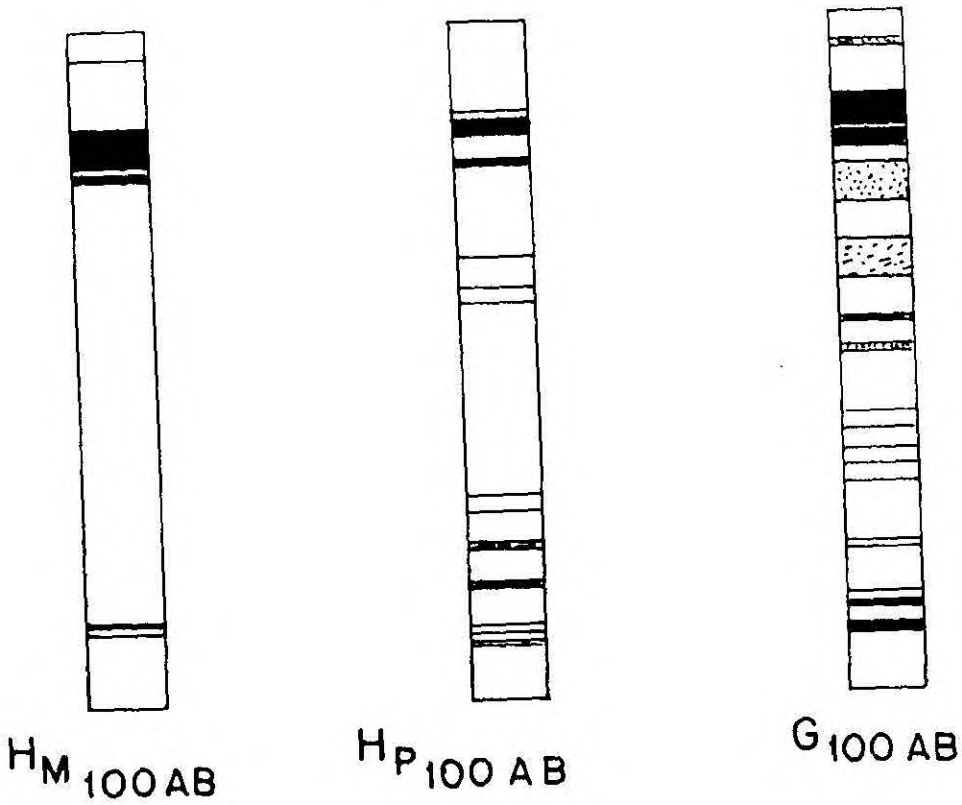


FIG. 4.

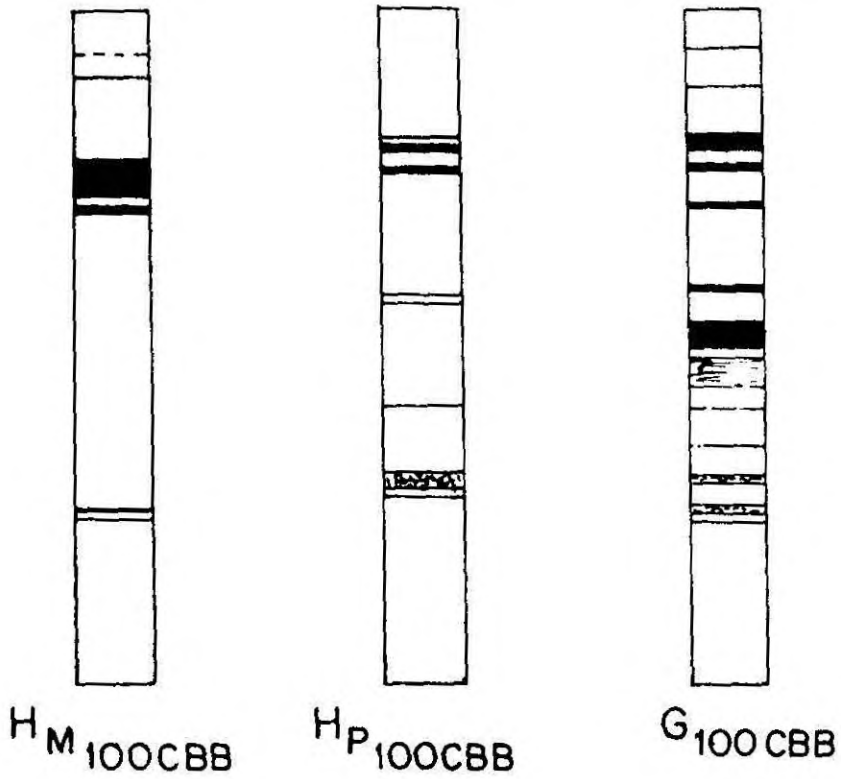
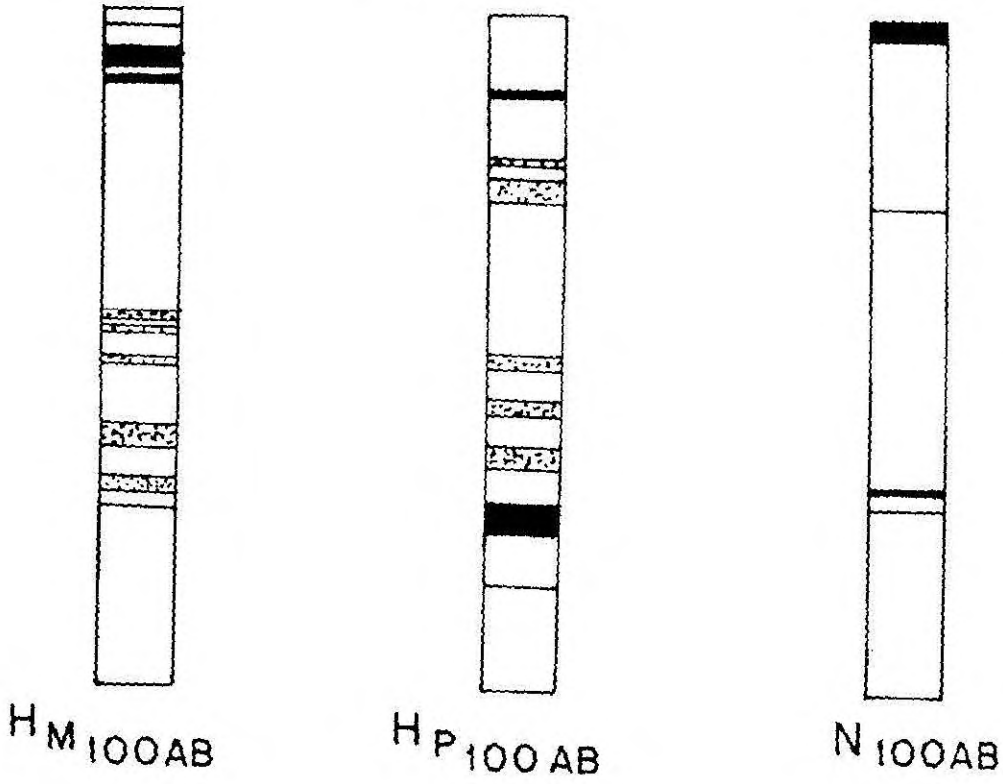


FIG. 5.



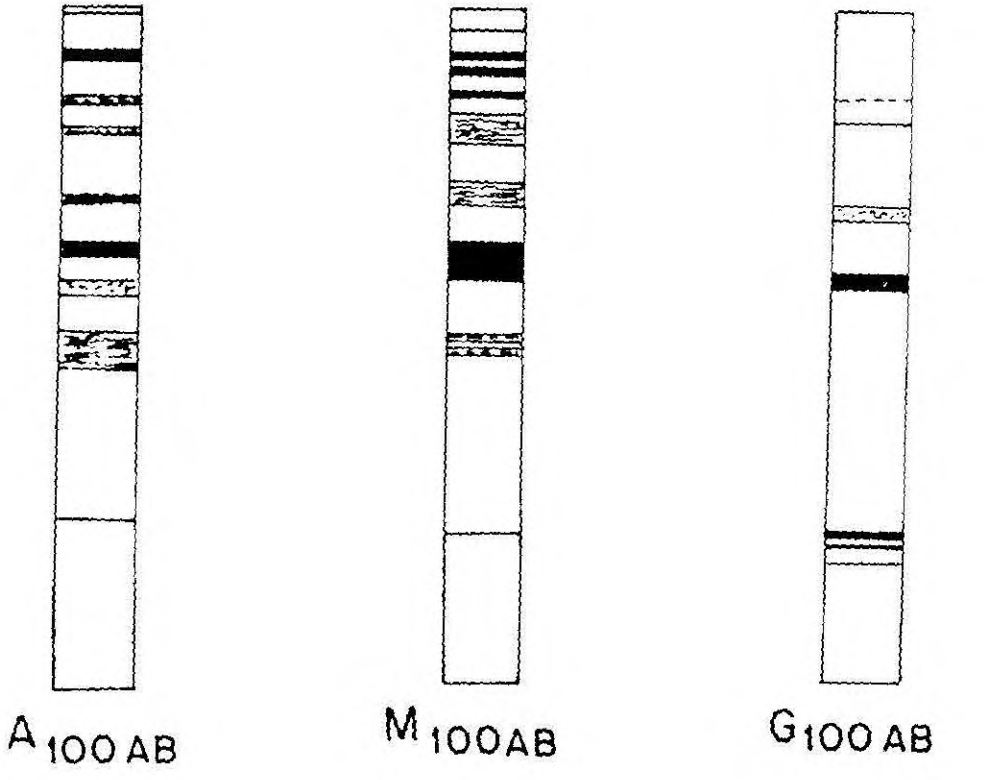
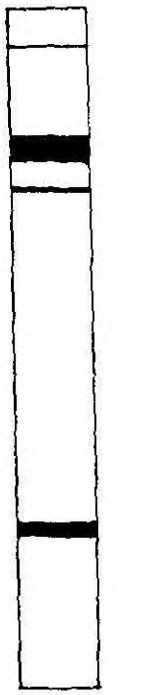
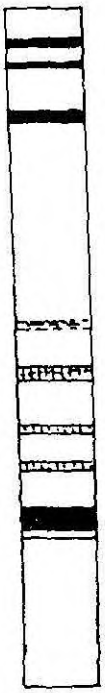


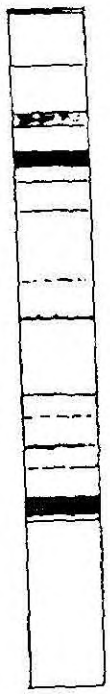
FIG. 6



$HM_{100\,AB}$

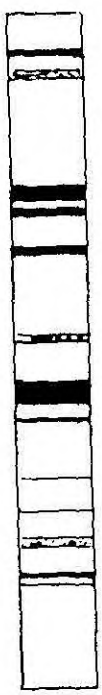


$HP_{100\,CBB}$

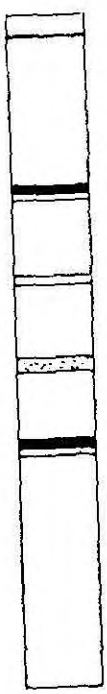


$G_{100\,AB}$

$A_{100\,AB}$



$M_{100\,AB}$

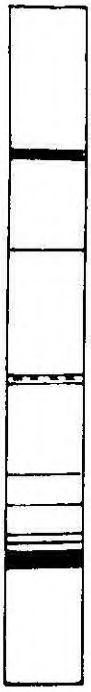


$N_{100\,AB}$

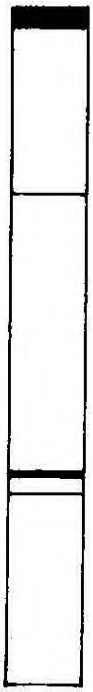
FIG. 7.

$A_{100\,AB}$

$M_{100\,AB}$



$H_{P100\,AB}$



N_{100AB}

FIG. 8.

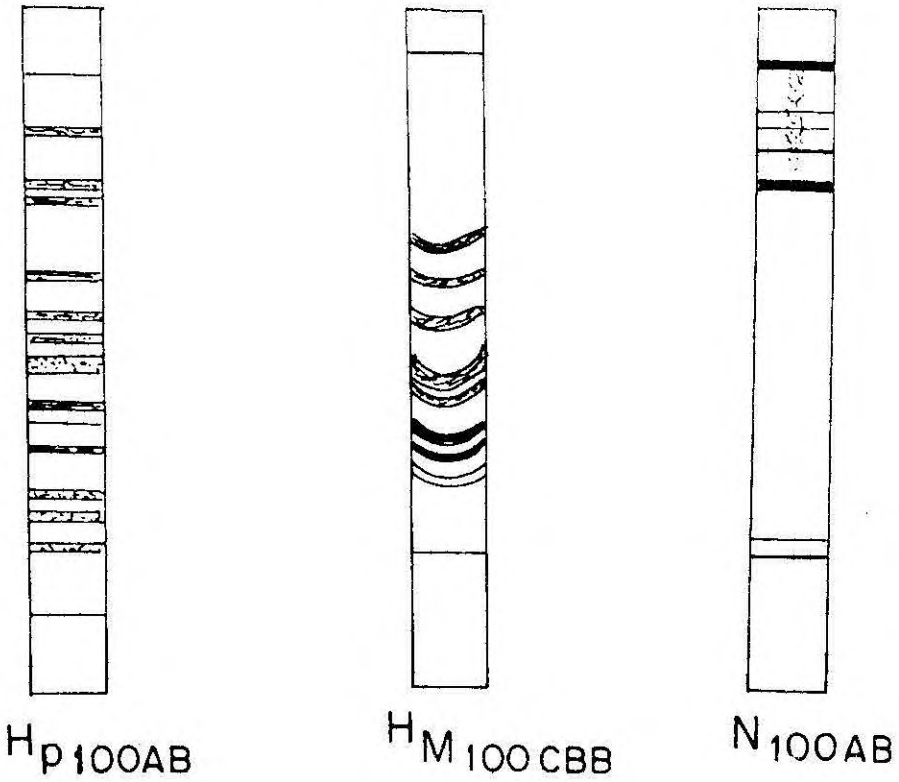
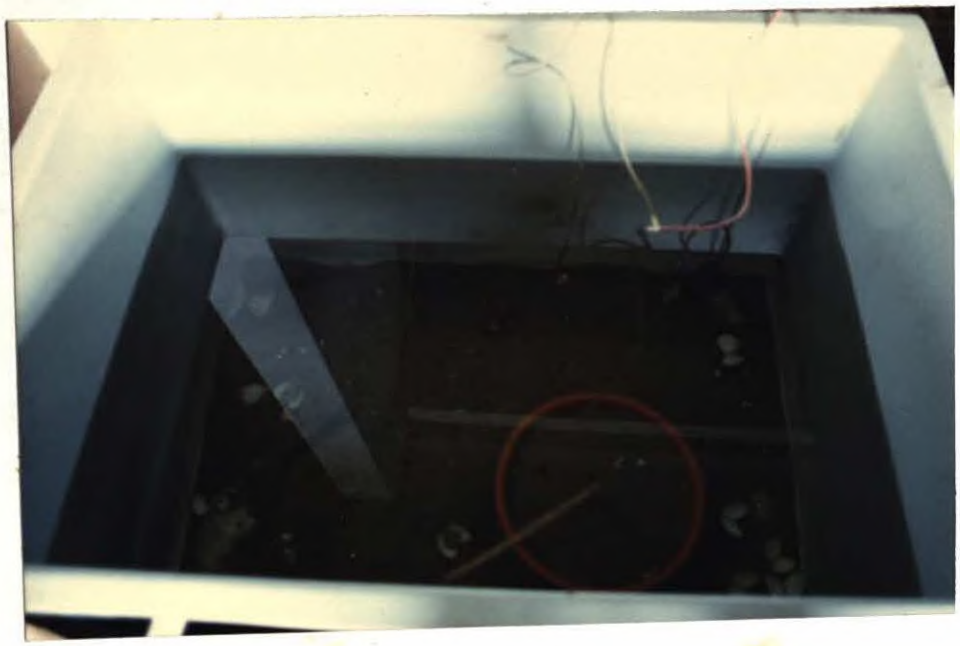


PLATE-1

1a WILD SPECIMEN

1b MATURATION TANK

1c ELECTROCAUTAERY APPARATUS




OFF
OFF
MADE IN INDIA

PLATE-2

2a UNILATERAL EYESTALK ABLATION EXPERIMENT

2b BILATERAL EYESTALK ABLATION EXPERIMENT

2c DISC ELECTROPHORESIS SET UP

PLATE-3

3a REMOVAL OF EXOSKELETON ALONG WITH THELYCUM AFTER EYESTALK ABLATION

3b CHECKING THE OVARY DEVELOPMENT AGAINST SUNLIGHT AFTER EYESTALK ABLATION

3c NEUROENDOCRINE SYSTEM OF EXPERIMENTAL ANIMAL

Plate - 3


OPTIC GANGLIA
CEREBRAL GANGLIA
SINUS GLAND
CIRCUM OESOPHAGEAL CONNECTIVE
SUB OESOPHAGEAL GANGLIA
THORACIC GANGLIA
ABDOMINAL GANGLIA
NEUROENDOCRINE SYSTEM OF Penaeus indicus H.Milne Edwards

PLATE-4. STANDARDISATION OF METHODOLOGY

4a. 5%(A) + 2%(B) GEL

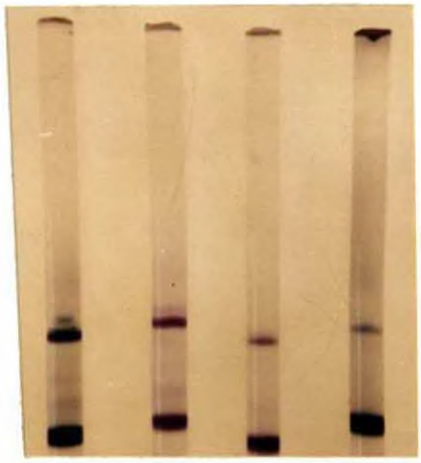
I	II
1. HP50AB	1. E100CBB
2. HP50CBB	2. E100AB
3. HP75CBB	3. HM50AB
4. HP75AB	4. HM50CBB

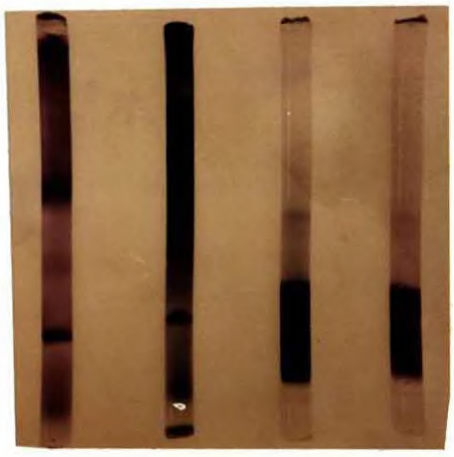
4b. 7%(A) + 2%(B) GEL

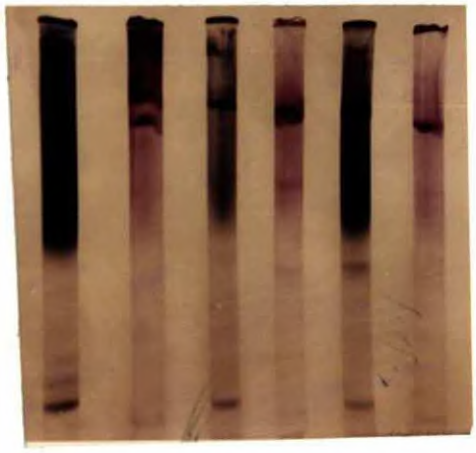
I	II
1. E100AB	1. G100AB
2. E100CB	2. G100CBB
3. E50AB	3. HP100AB
4. E50CBB	4. HP100CBB
5. N100AB	5. HM100AB
6. N100CBB	6. HM100CBB

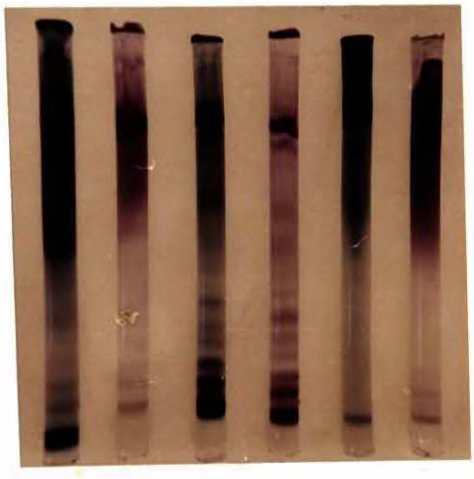
4c. 9%(A) + 2%(B) GEL

I	II
1. G100AB	1. E100AB
2. G100CBB	2. E100CBB
3. HP100AB	3. E50AB
4. HP100CBB	4. E50CBB
5. HM100AB	5. N100AB
6. HM100CBB	6. N100CBB

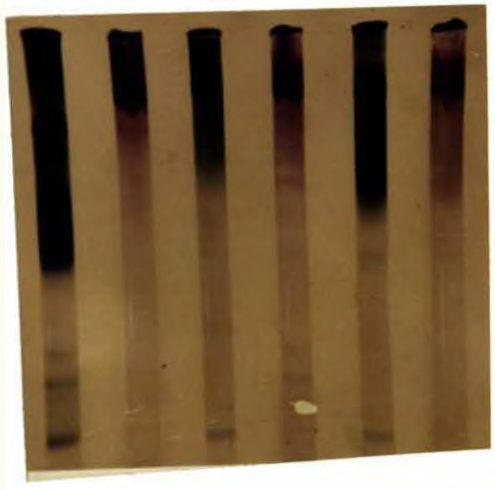








II



I

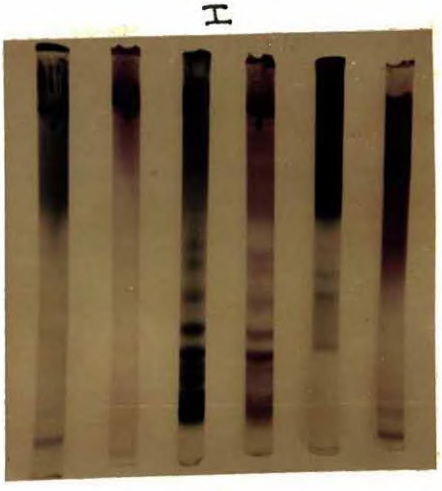


PLATE-5. BEFORE EYESTALK ABLATION IN 10%(A) + 2%(B) GEL

5a.

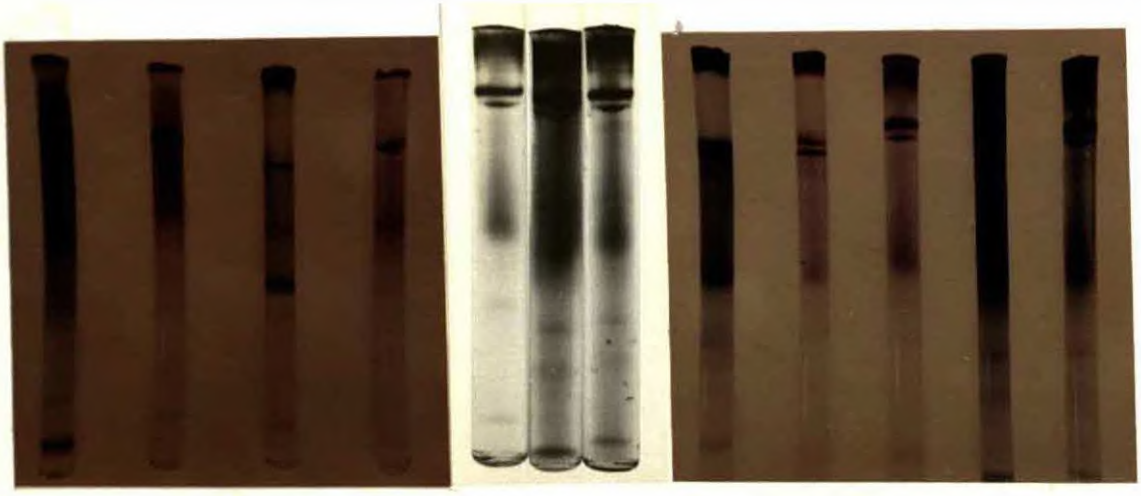
I	II	III
1. G100AB	1. HM100CBB	1. E100AB
2. G100CBB	2. HM100AB	2. E100CBB
3. N100AB	HM100AB	3. HM100CBB
4. N100CBB		4. HM100AB
		5. HM100AB

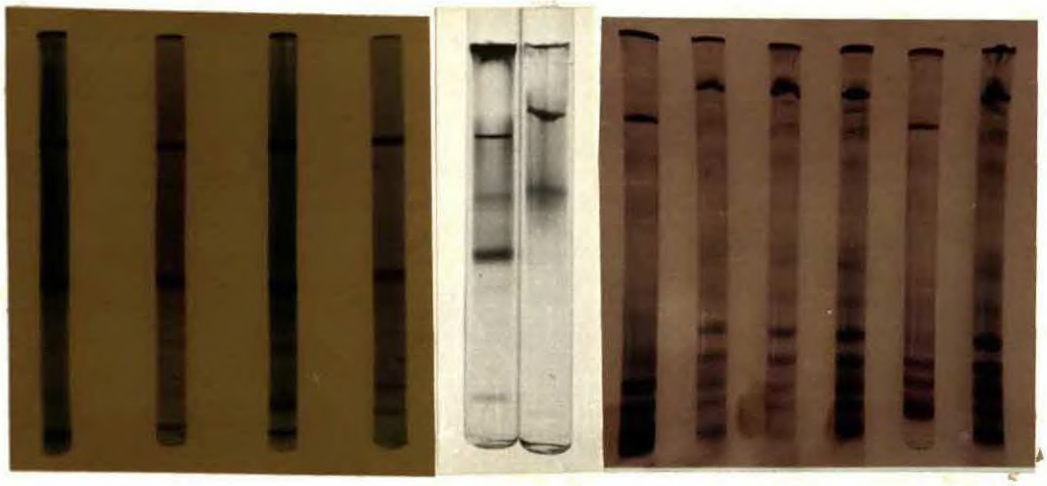
5b.

I	II	III
1. A100AB	1. N100AB	1. E100AB
2. A100CBB	2. N100CBB	2. HP100CBB
3. M100AB		3. HP100CBB
4. M100CBB		4. HP100AB
		5. HP100CBB
		6. HP100AB

5c.

I		II	III
1. A100AB	1. E100AB	1. G100AB	1. HP75AB
2. A100CBB	2. E100CBB	2. G100CBB	2. HP50AB
3. M100AB			3. HP100CBB
4. M100CBB			4. HP100AB
			5. HP100CBB
			6. HP100AB





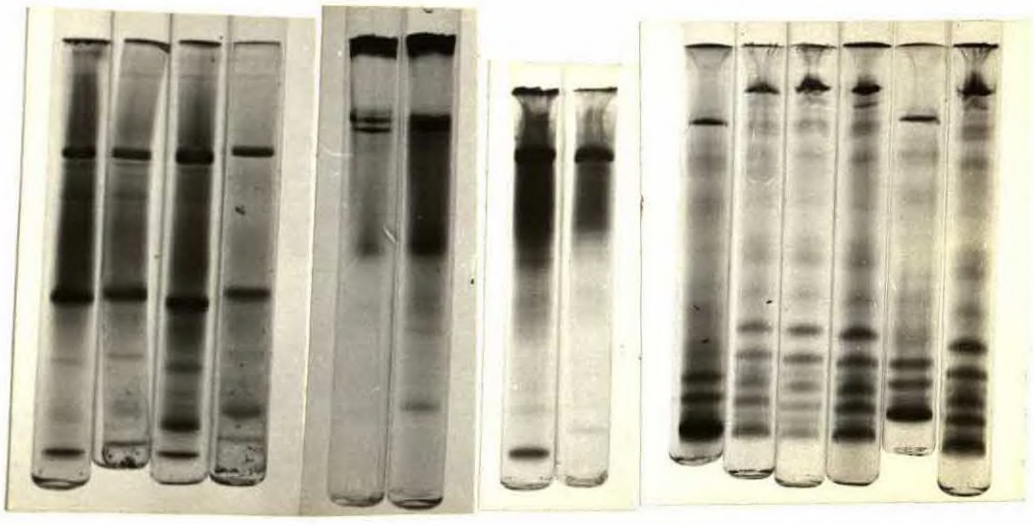


PLATE-6. AFTER UNILATERAL EYESTALK ABLATIONS IN 10%(A) + 2%(B) GEL

6a. (after 7 days)

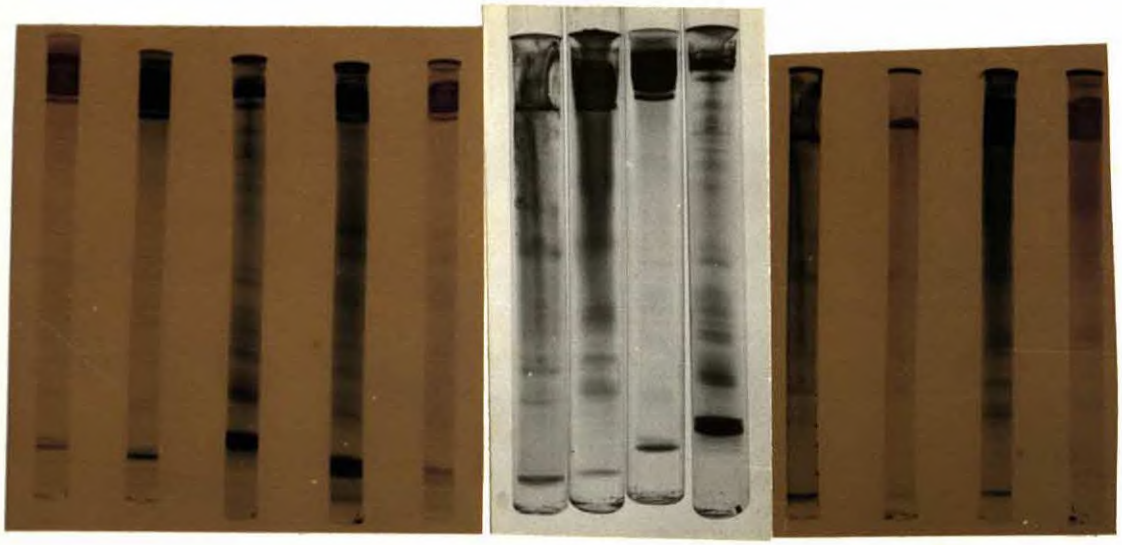
I	II	III
1. HM100CBB	1. N100AB	1. N100AB
2. HM100AB	2. E100AB	2. N100CBB
3. HP100AB	3. HM100AB	3. E100AB
4. G100AB		
5. G100CBB		

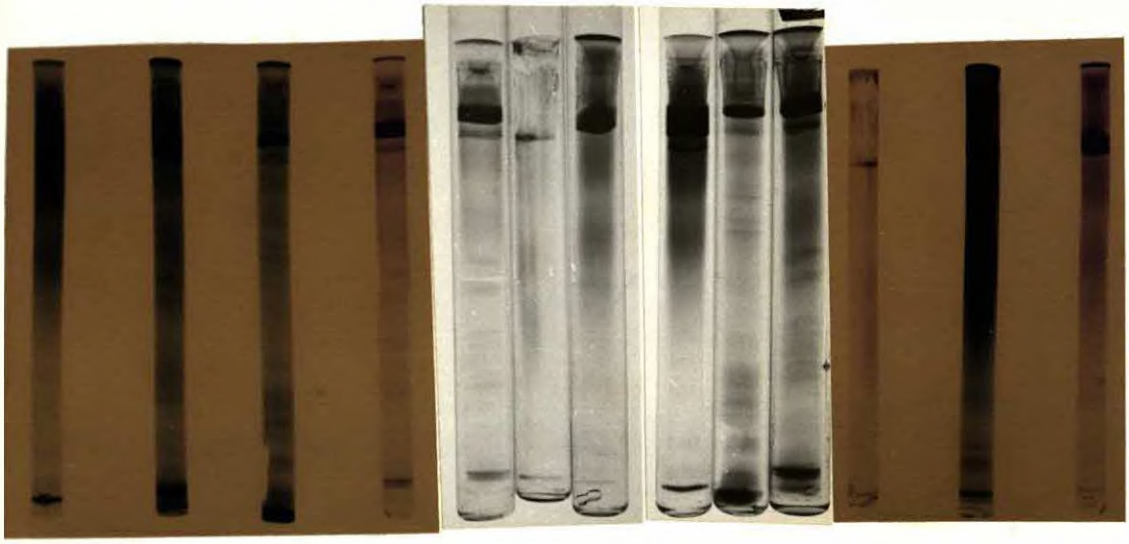
6b. (after days)

I	II	III	IV
1. HM100AB	1. G100CBB	1. HM100AB	1. N100CBB
2. HP100AB	2. N100CBB	2. HP100AB	2. E100AB
3. G100AB	3. E100CBB	3. G100AB	3. E100CBB
4. G100CBB			

6c. (after 30 days)

I	II	III	IV
1. N100AB	1. N100AB	1. HM100CBB	1. M100AB
2. A100CBB	2. HP100AB	2. HP100CBB	2. A100AB
3. M100AB	3. G100AB	3. G100CBB	3. M100CBB
			4. A100CBB





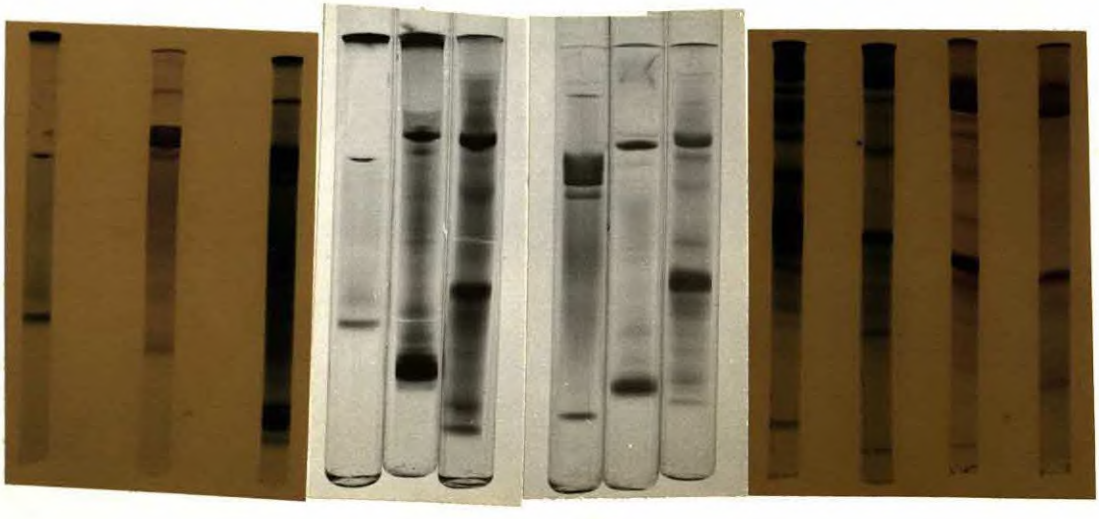
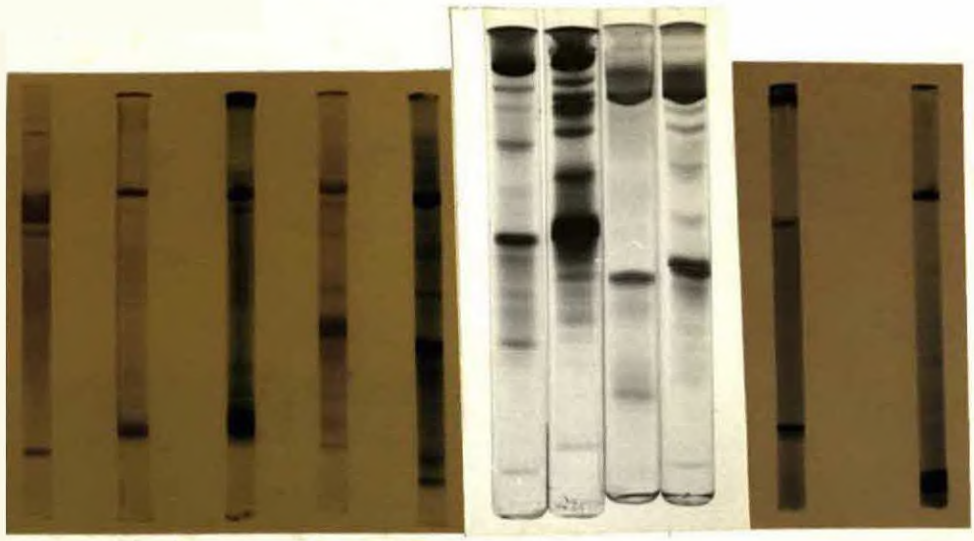
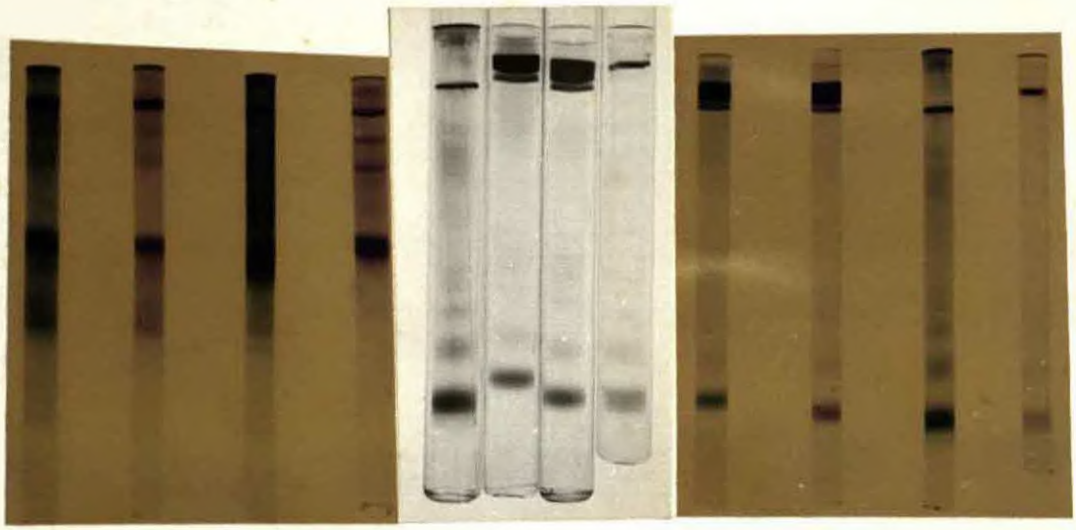


PLATE-7 AFTER UNILATERAL, BILATERAL EYESTALK ABLATION AND WILD SPECIMEN IN 10%(A) + 2%(B) GEL.

	I	II	III
7a. (after 30 days of unilateral (I & II) and wild specimen (III)	1. HM100CBB 2. HP100CBB 3. HP100AB 4. G100CBB 5. G100AB	1. A100AB 2. M100AB 3. A100CBB 4. M100CBB	1. E100AB 2. HP100AB
7b. (after 7 days of bilateral)	1. A100AB 2. A100CBB 3. M100AB 4. M100CBB	1. HP100AB 2. HM100AB 3. HM100CBB 4. HP100CBB	1. HM100AB 2. HM100CBB 3. HP100AB 4. HP100CBB
7c (after 7 days (II) and 15 days of bilateral)	1. A100AB 2. A100CBB 3. M100AB 4. M100CBB	1. A100AB 2. A100CBB 3. M100AB 4. M100CBB	1. HM100AB 2. HM100CBB 3. HP100CBB





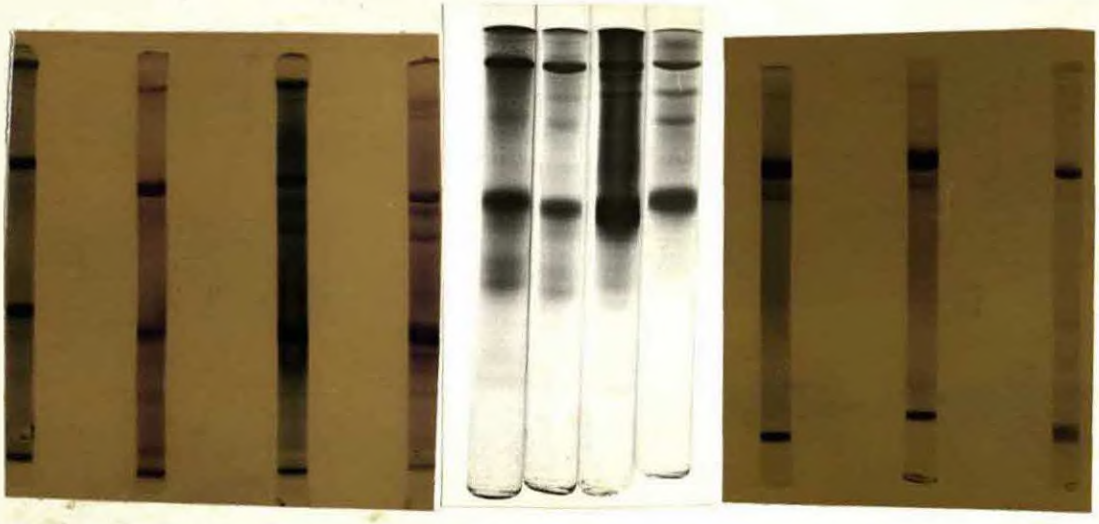


PLATE-8. AFTER BILATERAL EYESTALK ABLATION IN 10%(A) + 2%(B) GEL

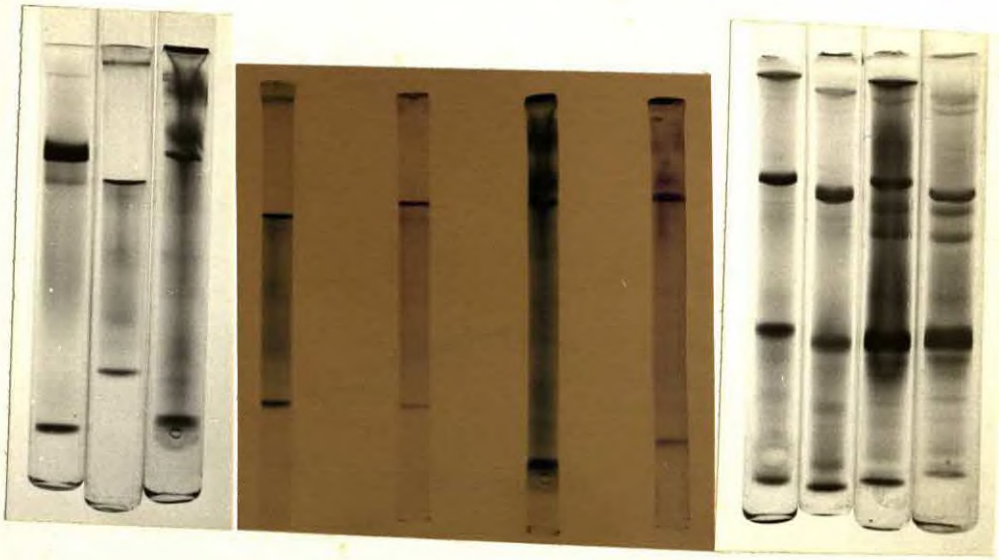
	I	II	III
8a. (after 15 days)	1. HM100AB 2. N100AB 3. G100AB	1. N100AB 2. N100CBB 3. G100AB 4. G100CBB	1. A100AB 2. A100CBB 3. M100AB 4. M100CBB

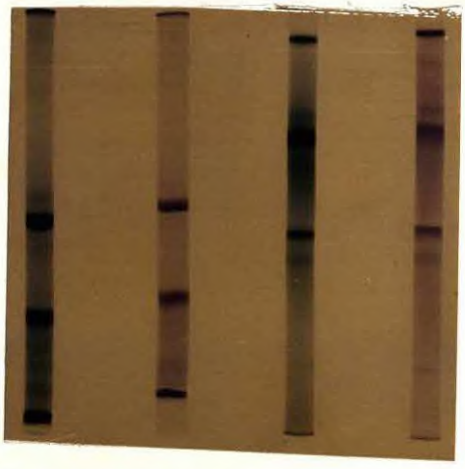
8b. (after 30 days)

1. A100AB
2. A100CBB
3. M100AB
4. M100CBB

8c. (after 30 days)

1. N100AB
2. HP100AB
3. A100AB
4. A100CBB
5. M100AB
6. M100CBB
7. G100AB
8. G100CBB





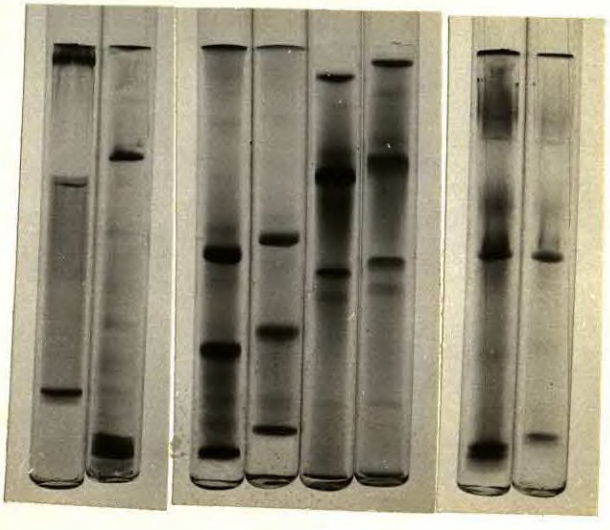


TABLE-1. DETAILS OF GEL COMBINATION

Acrylamide (A)		Bisacrylamide (B)	Double Distilled Water (DDW)	Gel Buffer (Tris-HCl.)	Ammonium Persulfate	Total
10%	5 ml	5% - 5 ml	-	5 ml	5 ml	------ 20 ml for 12 gels ------
	5 ml	4% - 4 ml	1 ml	5 ml	5 ml	
	5 ml	3% - 3 ml	2 ml	5 ml	5 ml	
	5 ml	2% - 2 ml	3 ml	5 ml	5 ml	
9%	4.5 ml	5% - 5 ml	0.5 ml	5 ml	5 ml	
	4.5 ml	4% - 4 ml	1.5 ml	5 ml	5 ml	
	4.5 ml	3% - 3 ml	2.5 ml	5 ml	5 ml	
	4.5 ml	2% - 2 ml	3.5 ml	5 ml	5 ml	
7%	3.5 ml	5% - 5 ml	1.5 ml	5 ml	5 ml	
	3.5 ml	4% - 4 ml	2.5 ml	5 ml	5 ml	
	3.5 ml	3% - 3 ml	3.5 ml	5 ml	5 ml	
	3.5 ml	2% - 2 ml	4.5 ml	5 ml	5 ml	
5%	2.5 ml	5% - 5 ml	2.5 ml	5 ml	5 ml	
	2.5 ml	4% - 4 ml	3.5 ml	5 ml	5 ml	
	2.5 ml	3% - 3 ml	4.5 ml	5 ml	5 ml	
	2.5 ml	2% - 2 ml	5.5 ml	5 ml	5 ml	

TABLE-3. DETAILS OF GENERAL PROTEIN BANDS IN DIFFERENT TISSUES BEFORE EYESTALK ABLATION

Name of tissue sample (1)	Movement of marker dye in mm (2)	Band patterns								Total (11)
		1xcl (3)	2xcl (4)	3xcl (5)	4xcl (6)	1xdf (7)	2xdf (8)	3xdf (9)	4xdf (10)	
HM100AB	71	-	1	2	-	-	1	-	1	5
HP100AB	75	7	1	1	6	-	1	-	-	16
G100AB	68	3	1	1	1	-	-	-	1	7
E100AB	70	-	-	2	1	3	1	-	-	7
N100AB	68	-	1	1	1	-	1	-	-	4
A100AB	76	3	-	2	2	3	1	-	-	11
M100AB	77	4	1	2	2	2	-	1	-	12

TABLE-2. DETAILS OF LENGTH, WEIGHT AND MATURITY STAGES OF EXPERIMENTAL ANIMALS OF *P. INDICUS* AT THE END OF EACH EXPERIMENTAL DAYS

Specimen characters	Before Eyestalk ablation	Unilateral eye-stalk ablation			Bilateral eye-stalk ablation			Spawned specimen	Wild specimen
		7th day	15th day	30th day	7th day	15th day	30th day		
Length in mm	136	142	136	138	131	137	134	140	133
Weight in gm	19	22	20	21	18	19	18	20.5	18
Maturity stages	Immature with thin translucent ovary	Middle lobes of ovary spreaded	Posterior lobe of ovary developed	ovary remained as such without further development	Ovary in early maturing stage	ovary in late maturing stage	Ovary remained as such	Shrunken ovary	Ovary in late maturing stage

TABLE-3. DETAILS OF GENERAL PROTEIN BANDS IN DIFFERENT TISSUES BEFORE EYESTALK ABLATION

Name of tissue sample (1)	Movement of marker dye in mm (2)	Band patterns								Total (11)
		1xcl (3)	2xcl (4)	3xcl (5)	4xcl (6)	1xdf (7)	2xdf (8)	3xdf (9)	4xdf (10)	
HM100AB	71	-	1	2	-	-	1	-	1	5
HP100AB	75	7	1	1	6	-	1	-	-	16
G100AB	68	3	1	1	1	-	-	-	1	7
E100AB	70	-	-	2	1	3	1	-	-	7
N100AB	68	-	1	1	1	-	1	-	-	4
A100AB	76	3	-	2	2	3	1	-	-	11
M100AB	77	4	1	2	2	2	-	1	-	12

TABLE-4. DETAILS OF GENERAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER UNILATERAL EYESTALK ABLATION

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
					AFTER 7 DAYS					
HM100AB	75	1	2	1	1	2	-	-	-	7
HP100AB	75	12	5	2	-	3	2	1	-	25
G100AB	80	2	3	2	1	5	3	1	-	17
E100CBB	79	1	3	1	1	-	2	-	-	8
N100AB	80	3	1	1	-	2	-	-	-	7
					AFTER 15 DAYS					
HM100AB	80	1	-	2	1	-	-	-	-	4
HP100AB	83	8	1	1	1	1	1	-	-	13
G100AB	82	8	-	3	1	-	3	2	-	17
E100CBB	81	5	-	-	1	-	-	-	-	6
N100AB	80	1	-	1	-	-	-	-	-	2
					AFTER 30 DAYS					
HM100CBB	68	1	2	1	-	1	-	-	-	5
HP100CBB	65	5	-	1	-	-	-	-	1	7
G100 CBB	68	3	1	3	-	2	3	-	-	12
N100AB	66	-	1	2	-	-	-	-	-	3
A100AB	80	6	3	1	1	-	-	-	-	11
M100AB	76	6	1	6	1	1	1	-	-	16

TABLE-5. DETAILS OF GENERAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER BILATERAL EYESTALK ABLATION

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
					AFTER 7 DAYS					
HM100AB	66	-	1	1	1	4	-	1	-	8
HP100AB	76	-	-	1	1	3	2	-	-	7
G100AB	75	1	1	1	1	1	1	-	-	6
N100AB	65	1	-	3	-	-	-	-	-	4
A100AB	68	1	-	-	2	2	2	-	1	8
M100AB	70	1	2	1	1	-	2	-	2	9
					AFTER 15 DAYS					
HM100AB	69	1	1	1	1	-	-	-	-	4
HP100CBB	70	3	1	1	-	1	2	-	-	8
G100AB	68	5	-	2	-	3	2	1	-	13
N100AB	59	3	1	2	-	-	1	-	-	7
A100AB	76	3	-	2	2	3	4	-	-	14
M100AB	76	4	3	3	1	1	-	-	-	12
					AFTER 30 DAYS					
HP100AB	76	3	-	4	-	-	1	-	-	8
N100AB	65	1	-	2	-	-	-	-	-	3
A100AB	72	1	2	2	1	1	2	-	-	9
M100AB	74	-	-	-	3	3	3	-	-	9

TABLE-6. DETAILS OF GENERAL PROTEIN BANDS IN DIFFERENT TISSUES OF WILD SPECIMEN AND SPAWNED SPECIMEN.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
					WILD SPECIMEN					
HM100CBB	72	5	1	-	-	-	1	2	1	10
HP100AB	80	2	3	1	-	8	-	-	-	14
G100AB	70	4	1	1	-	-	-	2	-	8
E100AB	70	1	-	1	-	1	1	-	-	4
N100AB	72	3	-	1	1	-	1	-	-	6
					SPAWNED SPECIMEN					
HM100AB	66	-	1	1	1	-	-	-	-	3
HP100AB	65	3	1	1	1	-	-	-	1	7
G100AB	65	4	1	1	1	-	1	-	1	9
E100AB	66	1	2	-	1	-	-	-	1	5
N100AB	65	-	-	1	-	1	1	1	-	4
A100AB	65	3	2	1	2	1	1	-	1	10
M100AB	65	-	4	3	2	-	-	-	1	10

TABLE-7. A COMPARISON OF TOTAL NUMBER OF GENERAL PROTEIN BANDS OF UNILATERAL, BILATERAL EYESTALK ABLATION EXPERIMENT, SPAWNED SPECIMEN AND WILD SPECIMEN.

Name of tissue sample	Before eyestalk ablation	Unilateral eyestalk ablation			Bilateral eyestalk ablation			Spawned specimen (a)	Wild specimen (b)
		7 days	15 days	30 days	7 days	15 days	30 days		
Hemolymph (HM)	5	7 (5)	4	5 (5)	8 (5)	4 (5)	NA	3	10
Hepatopancreas (HP)	16	25(15)	13	7 (17)	7(15)	8(17)	8	7	14
Ovary (G)	7	17 (6)	17	12 (7)	6 (6)	13 (7)	NA	9	8
Eye (E)	7	8 (6)	6	NA (7)	*	*	*	5	4
Nervous System (N)	4	7 (4)	2	3 (4)	4 (4)	7 (4)	3	4	6
Antena base (A)	11	NA	NA	11(10)	8(NA)	14(10)	9	10	NA
Body Muscle (M)	12	NA	NA	16(12)	9(NA)	12(12)	9	10	NA

N.B.
- NA - Not available
- * - Due to bilateral eyestalk ablation both eyes are removed
- a - Unilaterally eyestalk ablated spawned specimen on 6th day.
- b - Wild specimen of late maturing stage collected from open sea and after 3 days of unilateral eyestalk ablation.
- ( ) - Protein patterns of control (Zero day)

TABLE-8. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES BEFORE EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	HEMOLYMPH (HM100AB)	
1	0 - 1	2xcl
2	17 - 18	2xdf
3	30 - 36	4xdf
4	56 - 57	3xcl
5	58 - 61	3xcl
	HEPATOPANCREAS (HP100AB)	
1	0 - 2	4xcl
2	4	1xcl
3	7 - 8	4xcl
4	10 - 12	4xcl
5	13	1xcl
6	15 - 17	4xcl
7	19 - 21	4xcl
8	25 - 26	2xdf
9	33 - 34	1xcl
10	36 - 37	1xcl
11	45 - 46	1xcl
12	53 - 54	2xcl
13	58 - 59	3xcl
14	61	1xcl
15	63 - 64	1xcl
16	66 - 69	4xcl

TABLE-9. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES BEFORE EYESTALK ABLATION

I	II	III
	EYE (E100AB)	
1	15 - 16	1xdf
2	18 - 19	1xdf
3	20 - 21	1xdf
4	30 - 33	2xdf
5	53 - 54	3xcl
6	54 -- 55	3xcl
7	67 -- 70	4xcl
	OVARY (G100AB)	
1	0 - 1	3xcl
2	6	1xcl
3	11	1xcl
4	28 - 29	2xcl
5	41 - 53	4xdf
6	54 - 56	4xcl
7	59	1xcl
	NERVOUS SYSTEM (N100AB)	
1	0 - 1	2xcl
2	27 - 30	4xcl
3	39 - 40	2xdf
4	50 - 51	3xcl

TABLE-10. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES BEFORE EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	ANTENNA BASE (A100AB)	
1	1 - 2	3xcl
2	5	1xdf
3	9	1xdf
4	17 - 18	2xdf
5	26	1xdf
6	29 - 31	4xcl
7	47 - 48	3xcl
8	52	1xcl
9	54	1xcl
10	55 - 56	4xcl
11	70	1xcl
	BODY MUSCLE(M100AB)	
1	2	3xcl
2	6 - 7	3xcl
3	8	1xdf
4	10	1xdf
5	13	1xcl
6	17 - 18	2xcl
7	24 - 25	3xdf
8	28 - 30	4xcl
9	48	1xcl
10	54	1xcl
11	56 - 58	4xcl
12	70	1xcl

TABLE-11. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 7 DAYS OF UNILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	HEMOLYMPH (HM100AB)	
1	0 - 1	2xcl
2	2	2xcl
3	29 - 30	1xdf
4	32 - 33	1xdf
5	63 - 64	3xcl
6	65 - 71	4xcl
7	74	1xcl
	HEPATOPANCREAS (HP100AB)	
1	1	1xcl
2	2	1xcl
3	4	3xcl
4	5 - 6	3xcl
5	13	2xcl
6	14	2xcl
7	18	1xdf
8	19	1xdf
9	21	1xcl
10	22	1xcl
11	23	1xcl
12	25	1xcl
13	33	2xcl
14	36	1xcl
15	48 - 49	2xdf
16	50 - 51	2xcl
17	53	1xcl
18	55	1xcl
19	57	1xcl
20	58	1xcl
21	59	1xdf
22	61	2xdf
23	64	1xcl
24	66	2xcl
25	68 - 70	3xdf

TABLE-12. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 7 DAYS OF UNILATERAL EYESTALK ABLATION

Ban No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	EYE (E100AB)	
1	1 - 2	3xcl
2	14 - 16	2xcl
3	20 - 21	2xcl
4	28 - 30	2xcl
5	43 - 45	2xdf
6	51 - 53	2xdf
7	65	1xcl
8	66 - 74	4xcl
	OVARY (G100AB)	
1	1	1xcl
2	2	1xdf
3	5	2xcl
4	6	3xcl
5	15	2xcl
6	18	2xdf
7	23	2xdf
8	28	1xdf
9	34	1xcl
10	35 - 40	2xdf
11	44 - 45	2xdf
12	49 - 50	2xcl
13	60 - 61	1xdf
14	65	1xdf
15	67 - 68	1xdf
16	69 - 77	4xcl
17	78 - 80	3xcl
	NERVOUS SYSTEM (N100AB)	
1	1 - 2	3xcl
2	15	1xcl
3	16	1xdf
4	20	1xcl
5	41 - 42	2xdf
6	50	1xcl
7	67 - 68	2xcl

TABLE-13. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 15 DAYS OF UNILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	HEMOLYMPH (HM100AB)	
1	1 - 2	3xcl
2	60 - 61	3xcl
3	62 - 67	4xcl
4	76	1xcl
	HEPATOPANCREAS (HP100AB)	
1	1	3xcl
2	2	1xcl
3	3	1xcl
4	8 - 9	1xdf
5	13 - 14	2xdf
6	18	1xcl
7	20	1xcl
8	46	1xcl
9	48	1xcl
10	52	1xcl
11	64 - 65	2xcl
12	68 - 70	4xcl
13	71	1xcl

TABLE-14. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS OF AFTER 15 DAYS UNILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	OVARY (G100AB)	
1	0 - 1	3xcl
2	3 - 4	3xcl
3	5	1xcl
4	11	1xcl
5	12	1xcl
6	20	1xcl
7	22	1xcl
8	24	1xcl
9	27	1xcl
10	29	1xcl
11	37 - 38	2xdf
12	41 - 42	3xdf
13	47 - 52	2xdf
14	57 - 62	2xdf
15	65 - 67	3xcl
16	68 - 72	4xcl
17	78 - 79	3xdf
	EYE(E100CBB)	
1	0 - 1	1xcl
2	64 - 68	4xcl
3	71	1xcl
4	75	1xcl
5	77	1xcl
6	78	1xcl
	NERVOUS SYSTEM (N100AB)	
1	0 - 1	1xcl
2	61 - 62	3xcl

TABLE-15. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 30 DAYS OF UNILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	HEMOLYMPH (HM100CBB)	
1	1	2xcl
2	41 - 42	2xcl
3	43 - 48	3xcl
4	59	1xcl
5	62	1xcl
	HEPATOPANCREAS(HP100CBB)	
1	1 - 3	4xdf
2	12	1xcl
3	26	1xcl
4	27	1xcl
5	43 - 44	1xcl
6	46 - 47	3xcl
7	48	1xcl

TABLE-16. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 30 DAYS OF UNILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
	OVARY (G100CBB)	
1	1 - 2	2xdf
2	5 - 6	2xdf
3	10	1xdf
4	15	1xdf
5	18 - 22	2xdf
6	23 - 27	3xcl
7	31 - 32	2xcl
8	42 - 43	1xcl
9	47 - 48	2xcl
10	50 - 52	3xcl
11	58	1xcl
12	68	1xcl
	NERVOUS SYSTEM (N100AB)	
1	12 - 13	3xcl
2	43	2xcl
3	65 - 66	3xcl

TABLE-17. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 30 DAYS OF UNILATEAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	ANTENNA BASE(A100AM)	
1	1	1xcl
2	24 - 25	2xcl
3	30	1xcl
4	34	1xcl
5	39	1xcl
6	42 - 43	3xcl
7	59 - 60	2xcl
8	66	1xcl
9	69 70	2xcl
10	72 - 77	4xcl
11	78 - 80	1xcl
	BODY MUSCLE (M100AB)	
1	2	1xcl
2	21	1xdf
3	22 - 24	2xdf
4	30	1xcl
5	32	1xcl
6	38	1xcl
7	39 - 42	4xcl
8	50 - 51	3xcl
9	57 - 58	3xcl
10	60 - 61	3xcl
11	62 - 63	2xcl
12	64 - 65	3xcl
13	67 - 68	3xcl
14	69 - 71	3xcl
15	73	1xcl
16	74 - 76	1xcl

TABLE-18. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 7 DAYS OF BILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	HEMOLYMPH (HM100AM)	
1	2 - 4	3xdf
2	8 - 11	1xdf
3	19 - 2-	1xdf
4	23 - 24	1xdf
5	25 - 26	1xdf
6	56 - 57	3xcl
7	58 - 61	4xcl
8	64	2xcl
	HEPATOPANCREAS (HP100AB)	
1	7 - 11	4xcl
2	16 - 19	2xdf
3	23 - 25	2xdf
4	29 - 31	1xdf
5	51 - 54	1xdf
6	56 - 57	1xdf
7	65 - 66	3xcl
	NERVOUS SYSTEM (N100AB)	
1	2 - 3	3xcl
2	40	1xcl
3	62 - 65	3xcl

TABLE-19. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 7 DAYS OF BILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	OVARY (G100AB)	
1	2	2xcl
2	3 - 4	3xcl
3	37 - 39	4xcl
4	46 - 48	2xdf
5	59	1xcl
6	62	1xcl
	ANTENNA BASE(A100AB)	
1	20 - 25	4xdf
2	30 - 32	4xdf
3	35 - 37	4xcl
4	42 - 43	1xdf
5	51 - 52	2xdf
6	55 - 56	1xdf
7	61 - 62	4xcl
8	67 - 68	1xcl
	BODY MUSCLE (M100AB)	
1	24 - 25	2xdf
2	26 - 27	2xdf
3	34 - 39	4xcl
4	44 - 47	4xdf
5	52 - 56	4xdf
6	58 - 59	2xcl
7	61 - 62	2xcl
8	63 - 64	2xcl
9	67	1xcl

TABLE-20. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 15 DAYS OF BILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	HEMOLYMPH (HM100AB)	
1	0 - 1	3xcl
2	45	2xcl
3	49 - 52	4xcl
4	64	1xcl
	HEPATOPANCREAS(HP100AM)	
1	1 - 4	2xcl
2	9 - 10	1xcl
3	14 - 15	1xdf
4	21 - 23	2xdf
5	28 - 29	2xdf
6	55 - 57	3xcl
7	62 - 63	1xcl
8	65 - 66	1xcl

TABLE-21. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 15 DAYS OF BILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	NERVOUS SYSTEM (N100AB)	
1	1 - 1	3xcl
2	11 - 13	2xdf
3	23	1xcl
4	24	1xcl
5	34	1xcl
6	35 - 36	3xcl
7	56	2xcl
	OVARY (G100AB)	
1	1 - 3	3xcl
2	7	1xdf
3	10	2xdf
4	14	1xdf
5	17	1xcl
6	27	2xdf
7	32	1xdf
8	41	1xcl
9	45	1xcl

TABLE-22. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 15 DAYS OF BILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	ANTENNA BASE (A100AB)	
1	1 - 2	3xcl
2	16 - 18	2xdf
3	20 - 21	2xdf
4	24 - 25	2xdf
5	27 - 30	4xcl
6	32 - 34	1xdf
7	47 - 49	2xdf
8	50	1xcl
9	53 - 55	4xcl
10	69	1xdf
11	70	1xdf
12	72	3xcl
13	75	1xcl
	BODY MUSCLE (M100AB)	
1	1 - 2	3xcl
2	5 - 6	1xdf
3	10	1xcl
4	14	1xcl
5	22	2xcl
6	24 - 27	4xcl
7	32 - 33	1xcl
8	44 - 45	2xcl
9	49 - 50	2xcl
10	51 - 52	3xcl
11	67 - 68	1xcl
12	70 - 71	3xcl

TABLE-23. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES AFTER 30 DAYS OF BILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	II
	HEPATOPANCREAS (HP100AB)	
1	2 - 4	3xcl
2	5	3xcl
3	6	3xcl
4	10	1xcl
5	14	1xcl
6	26 - 27	2xdf
7	44	1xcl
8	51 - 52	3xcl
	NERVOUS SYSTEM (N100AB)	
1	2 - 3	3xcl
2	40	3xcl
3	62 - 65	3xcl

TABLE-24. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BAND IN DIFFERENT TISSUES AFTER 30 DAYS OF BILATERAL EYESTALK ABLATION

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	ANTENNA BASE (A100AB)	
1	7	2xcl
2	28	2xcl
3	29	1xcl
4	31 - 32	3xcl
5	40 - 46	2xdf
6	47 - 49	4xcl
7	51 - 52	2xdf
8	62	1xdf
9	66 - 67	3xcl
	BODY MUSCLE(N100AB)	
1	0 - 1	4xcl
2	4 - 6	2xdf
3	8 - 10	2xdf
4	12 - 14	2xdf
5	18 - 20	4xcl
6	35 - 37	4xcl
7	40	1xdf
8	41	1xdf
9	48 48	1xdf

TABLE-25. DETAILS OF DISTANCE MIGRATED BY INDIVIDUAL PROTEIN BANDS IN DIFFERENT TISSUES OF WILD SPECIMEN

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
I	II	III
	HEMOLYMPH (HM100AB)	
1	11	1xcl
2	12	1xcl
3	16 - 17	1xcl
4	18 -19	1xcl
5	22 -23	2xdf
6	24 - 27	4xdf
7	32 - 33	3xdf
8	36 - 37	1xcl
9	41 - 42	2xcl
10	66	3xdf
	HEPATOPANCREAS (HP100AB)	
1	9 - 10	1xdf
2	13 - 14	2xcl
3	15 - 16	1xdf
4	22 - 23	2xcl
5	26 - 27	2xcl
6	28 - 29	2xcl
7	32	1xdf
8	38	1xdf
9	40	1xdf
10	44 - 45	1xcl
11	54 - 55	1xcl
12	56 - 57	1xdf
13	61 - 62	1xdf
14	71 - 72	3xcl

TABLE-26. DETAILS OF DISTANCE MIGRATED BY INDIVIDUALS PROTEIN BANDS IN DIFFERENT TISSUES OF WILD SPECIMEN

Band No.	Distance travelled with reference to the marker dye as the base in mm	Visual colour intensity
	OVARY (G100AB)	
1	34 - 42	3xdf
2	43 - 45	1xcl
3	50	1xcl
4	57	2xcl
5	55	1xcl
6	62 - 63	3xcl
	EYE (E100AB)	
1	29 - 30	1xcl
2	36 - 37	1xdf
3	47	2xdf
4	57	3xcl
	NERVOUS SYSTEM (N)	
1	2	1xcl
2	48 - 49	4xcl
3	53	2xdf
4	56	1xcl
5	58	1xcl
6	64 - 56	3xcl

SUMMARY

1. Standardisation of electrophoresis methodology for separation and resolution of general proteins in different tissues showed that AB was a better staining agent than CBB for both major and minor bands, 10% (A) + 2% (B) gel combination produced better separation, 100 mg of tissue per 1 ml DDW as better ratio for tissue homoginization, 100 µl of tissue sample as standard volume for sample application and 10 minutes of staining as optimum time for revealing all major and minor protein bands.

2. The general protein patterns in different tissues like HM, HP, G, E, N, A & M was found to be tissue specific in terms of total number of bands, electrophoretic mobility and even in staining intensity before eyestalk ablation.

3. The unilateral eyestalk ablation produced a positive profound response by different tissues showing additional number of protein bands in all the tissues on 7th day of ablation. The maximum increase (143%) in the number of protein bands was showed by ovary (G) followed by Nervous tissue (N) (75%), Hepatopancreas (HP) (56%), Hemolymph (HM) (40%) and eye (E) (15%). The effect of unilateral eyestalk ablation on 15th day was of a negative nature on the number of protein bands obtained compared to that of 7th day except that of ovary which remained unchanged even

on 15th day. The total number of protein bands in different tissues was found considerably reduced on 30th day of unilateral eyestalk ablation compared to 15th, 7th and zero days of ablation.

4. The effects of bilateral eyestalk ablation on the general protein in P. indicus were mainly of a negative in nature compared to that of unilateral eyestalk ablation. The total number of protein bands in all the tissues except HM was found considerably reduced on 7th day compared to that of zero day. The total number of protein bands of different tissues on 7th day of bilateral eyestalk ablation was significantly different from that of 7th day of unilateral eyestalk ablation. Though the total number of protein bands of HM was found considerably reduced on 15th day of bilateral eyestalk ablation, the total number of protein bands of ovary was suddenly found increased by more than 100% on 15th day. Similar increase was also indicated by nervous tissue for the same period.

5. The analysis of the tissue protein patterns of spawned specimen on 6th day of unilateral eyestalk ablation showed a significantly different patterns compared to that of 7th day of unilateral eyestalk ablation for the same period. However the total number of protein bands of HP and nervous tissue of the spawned specimen and that of bilaterally ablated specimen on 7th day was identical.

6. The total number of protein bands in different tissues of late maturing wild P. indicus collected from open sea and unilaterally eyestalk

ablated was found significantly different from that of spawned specimen as well as from that of either unilaterally or bilaterally ablated specimen during the experiment.

7. The effects of eyestalk ablation on the quality of general proteins in terms of staining intensity and electrophoretic mobility of certain bands were considerable compared to that of before eyestalk ablation.

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